

VOLUME 30

SEPTEMBER 1952

NUMBER 9

Canadian Journal of Chemistry

Editor: LÉO MARION

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF CHEMISTRY

(Formerly Section B, Canadian Journal of Research)

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Canadian Journal of Chemistry

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 30

SEPTEMBER, 1952

NUMBER 9

LYCOCTONINE AND ITS OXIDATION PRODUCTS¹

BY O. E. EDWARDS AND LÉO MARION

ABSTRACT

The ultraviolet spectra, basic strengths, and N-alkyl groups of the aconite alkaloids are discussed. Pyropseudoaconine has been shown to contain a conjugated system. The formulation $C_{19}H_{19-21}(OH)_3(OCH_3)_4NC_2H_5$ has been confirmed for lycoctonine. Study of its oxidation products has shown that lycoctonine has a methylene group adjacent to the nitrogen and a primary hydroxyl group. The two remaining hydroxyls have been shown to be vicinal, with one probably secondary and the other tertiary. A carbinolamine structure is suggested for hydroxylycoctonine. The new bases, isolycoctonine, desoxylycoctonine, and des-(oxymethylene)-lycoctonine are described.

INTRODUCTION

Aconitum lycoctonum (31) and many *Delphinium* species (6, 11, 12, 24, 27, 30, 36, 37) have been shown to contain derivatives of the polyhydroxy base lycoctonine. This base was chosen for an attempt to elucidate the structure of the more highly hydroxylated aconite and delphinium alkaloids since it occurs in both genera, it has an empirical formula representative of the group (Table I), and it is relatively accessible.

TABLE I
MOST PROBABLE FORMULAE FOR THE HYDROLYTIC BASES

Aconine	$C_{19}H_{19}$	$(OH)_3(OCH_3)_4NC_2H_5$
Mesaconine	$C_{19}H_{19}$	$(OH)_3(OCH_3)_4NCH_3$
Hypaconine	$C_{19}H_{20}$	$(OH)_4(OCH_3)_4NCH_3$
Pseudoaconine	$C_{19}H_{20}$	$(OH)_4(OCH_3)_4NC_2H_5$
Bikhaconine	$C_{19}H_{21}$	$(OH)_3(OCH_3)_4NC_2H_5$
Delphonine	$C_{19}H_{21}$	$(OH)_3(OCH_3)_4NCH_3$
Lycoctonine	$C_{19}H_{19-21}$	$(OH)_3(OCH_3)_4NC_2H_5$

A number of empirical formulae have been assigned to lycoctonine, i.e., $C_{25}H_{39-41}O_7N$ (11, 31, 35, 36, 37), $C_{26}H_{33}O_5N$ (25), $C_{24}H_{41}O_7N$ (6), and $C_{26}H_{43}O_7N$ (30). The present work supports the C_{25} formulation, but the analyses do not make it possible to reach a decision between the H_{39} and H_{41} possibilities. If no double bonds are present the H_{39} formula requires a seven ring structure

¹ Manuscript received April 29, 1952.

Contribution from the Division of Chemistry, National Research Laboratories, Ottawa. Issued as N.R.C. No. 2801. Presented in part at the 3rd Seminar on the Chemistry of Natural Products, Fredericton, New Brunswick, July, 1951.

for the base. If the N-ethyl group is present as such (see below) and the hydroxymethyl and methoxyl groups are taken into account, only 18 carbons and the nitrogen are available to construct these rings, a most unusual possibility. It seems more probable that the H_{41} formulation is correct, since it brings lycoctonine into line with the assigned formulae for the other aconines, and if one double bond is present this requires only five rings in the structure.

Goodson's finding that ethyl iodide was formed in the Herzig-Meyer N-alkyl determination on lycoctonine (13) has now been confirmed by the present authors. Rabinovich and Konovalova (30) reported the formation of acetaldehyde on permanganate oxidation of a base which is probably lycoctonine, and the same observation was made by Yunusov and Abubakirov (36) concerning their *delsine*, which again is most likely identical with lycoctonine. Hence lycoctonine most probably contains an N-ethyl group.

Jacobs and Huebner (16, 19) questioned the existence of an N-alkyl group in the aconite alkaloids, and suggested a bridge to nitrogen or a labile C-alkyl as possible alternatives. However the weight of evidence indicates the presence of the N-alkyl group, at least in the more highly hydroxylated bases. For example, aconine hydriodide gives ethyl iodide when pyrolyzed (10, 17, 23). It and mesaconine give rise to ethylamine and methylamine respectively on alkali fusion (10, 17, 23) and to acetaldehyde and formaldehyde respectively on oxidation with potassium permanganate (23). Whereas aconitine has one C-methyl which is attributable to the N-ethyl group (10, 23) the lactam (oxonitine) derivable from both it and mesaconine no longer contains one (10). Oxonitine has one more active hydrogen than aconitine (presumably NH) (10) and on alkali fusion gives off ammonia (23) indicating that the N-alkyl group has been replaced by hydrogen. It seems most unlikely that such consistent fragments could have arisen from such widely different degradations unless the simple N-alkyls were present. It is difficult to reconcile the above facts with the C_{33} formulation of oxonitine put forth by Jacobs, Elderfield, and Craig (18). The N-alkyl group that they found in the base arising from the action of methanolic hydrogen chloride on oxonitine probably arose by alkylation of the decarboxylated substance through

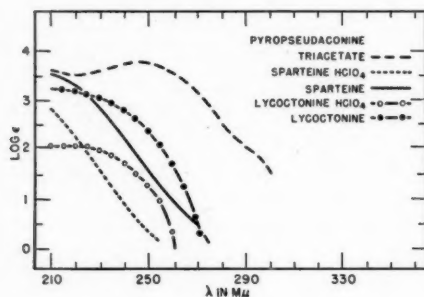


FIG. 1.

the action of methyl chloride formed under the reaction conditions. The presence of the group $>N-CH_2-CH_2OH$ in atisine has been postulated by Jacobs (21). This suggestion has not been confirmed, but could readily be discarded if the alkali fusion or permanganate oxidation of the base yielded ethylamine or acetaldehyde respectively. Neither of these methods is likely to produce an ethyl fragment from such a group.

At present there is no direct evidence for unsaturation in lycoctonine or the aconines in general. Lycoctonine shows only end absorption in the ultraviolet (Fig. 1) and in agreement with the findings of Rabinovich and Konovalova (30) the base and its simple transformation products are not hydrogenated in acid solution over Adams' catalyst. The pK of lycoctonine (8.8 in 50% aqueous methanol) is normal for a polycyclic base with no double bond near the nitrogen.

The alkamine nucleus of aconitine is inert to hydrogen over a platinum catalyst under conditions where the benzoyl group of the alkaloid is reduced to a hexahydrobenzoyl group (10, 18). However, Jacobs and co-workers (7) interpreted the ultraviolet spectra of aconine and delphonine as indicating the presence of conjugated unsaturation. Actually the spectra show no resemblance to those of conjugated dienes, and the error of their conclusion is borne out by a comparison of the spectrum of lycoctonine (similar to that of aconine) and its perchlorate with that of sparteine (no unsaturation) and its monoperchlorate (Fig. 1). The end absorption of the bases is due to the basic nitrogen, with some contribution from hydroxyls and methoxyls. An aminodiene system, suggested as a possibility by these workers, would have a spectrum similar to that of a conjugated triene, while vinylamines resemble conjugated dienes in their absorption characteristics (5), so neither of these possibilities need be considered for these bases.

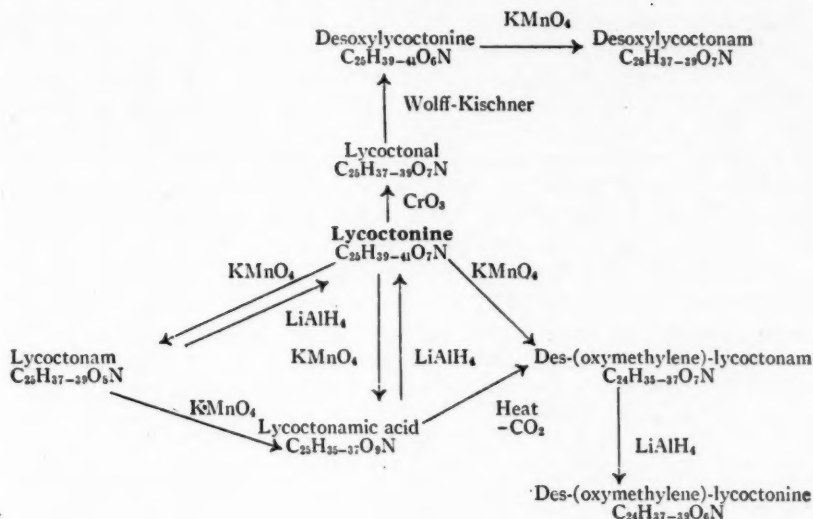
The nature of the "pyro" derivatives of the aconite alkaloids has been the subject of considerable speculation. Pyro- and isopyrodelphonine derivatives contain isolated double bonds arising from the loss of acetic acid (7, 20). The ultraviolet absorption spectrum of pyraconitine hydriodide in 95% ethanol has been found to have maxima nearly identical to those of aconitine hydriodide (λ_{max} 225 $m\mu$, $\log \epsilon$ 4.34; 274 $m\mu$, $\log \epsilon$ 2.97), hence no conjugated unsaturation results from the pyrolysis. In contrast to these, however, it has been found that pyropseudaconine triacetate has a spectrum corresponding to that of a conjugated diene (Fig. 1) (λ_{max} 246 $m\mu$, $\log \epsilon$ 3.78). It has a weak absorption peak in the infrared at 1634 cm^{-1} indicating a carbon-carbon double bond, which is absent in pseudaconine tetraacetate. This probably means that pseudaconine contains a completely substituted double bond, and that the pyro reaction introduces another in conjugation with it.

The pK of 10 (solvent?) given for delphonine (7) is high for a saturated polycyclic base, although simple cyclic tertiary amines have pK 's of the order of 11 in water (1-ethyl-2-methylpiperidine has pK 10.70 (1)). Hence this value seems to afford a weak basis for the suggestion (7) that delphonine is a tertiary vinylamine (1-ethyl-2-methyl-1,4,5,6-tetrahydropyridine has a pK in water of 11.57 (1)).

The lycoctonine "methiodide" reported in the literature (25, 31) is actually the hydriodide. This fact was also observed recently by Rabinovich and Konovalova (30). In attempts to carry out the Hofmann degradation of the supposed methiodide it was found that alkali liberated lycoctonine quantitatively, while silver oxide gave a new, less stable base. When lycoctonine hydriodide was prepared and found to be identical with the "methiodide", it became apparent that silver oxide had some action on lycoctonine. Actually in three hours at room temperature in aqueous methanol lycoctonine was oxidized by fresh silver oxide to a more water soluble base $C_{25}H_{39-41}O_8N$. Suginome and Ohno (35) have obtained the same compound. The markedly lower pK of the new base (5.6) in 50% aqueous methanol indicated a change in the vicinity of the nitrogen. The compound gave no red color with hot acetic anhydride and was recovered unchanged from aqueous sulphur dioxide solution, making it seem unlikely that it could be the N-oxide. Its infrared spectrum showed no carbonyl absorption, and its ultraviolet spectrum was uneventful. It was reduced slowly by hydrogen over Adams' catalyst in acid solution to a base $C_{25}H_{41}O_7N$, isomeric with lycoctonine. These facts, and the reported methyl ether formation with methanolic hydrogen chloride (35) all support the assumption that the oxidation product is a carbinolamine, analogous to pseudostrychnine. Like the latter it does not dehydrate readily to the vinylamine, and unlike berberine and cotarnine it does not form salts of anhydro quaternary character. This must mean that the N-ethyl group for steric reasons cannot approach the correct orientation for the formation of a $>C = \overset{+}{N} <$ structure, and that vinylamine formation is impossible either for steric reasons, or because the hydroxyl is adjacent to fully substituted carbons. The resistance of the base to further oxidation by silver oxide is sufficient proof that there is no hydrogen left on the carbon which is linked to the new hydroxyl. Suginome's name for the new base "hydroxylycoctonine" will be adopted, and the name isolycoctonine is suggested for its reduction product. The pK of the latter is only 6.7 in 50% aqueous methanol, suggesting even more serious steric hindrance of the nitrogen than pertains with lycoctonine.

Hydroxylycoctonine does not react appreciably with methanol, acetone, or nitromethane. A little neutral acetate is formed in $2\frac{1}{2}$ hr. at 110° with acetic anhydride, but only a trace of neutral product is obtained after 2.5 hr. in nitrous acid solution. The failure of these diagnostic tests for a carbinolamine can be attributed to the same cause as the extreme hindrance of the nitrogen. Hydroxylycoctonine is sensitive to heat, turning somewhat yellow when dried at 100° *in vacuo*.

The oxidation of aconite alkaloids with neutral potassium permanganate is reported to give lactams (18, 34). The action of permanganate in acetone containing a little acetic acid on lycoctonine yielded predominantly neutral products, of which two have been obtained crystalline. Among the acidic products was obtained a readily crystallizable lactam acid. The interrelationships between these compounds is shown below.



These reactions, together with the chromic acid oxidation of lycoctonine to the aldehyde, lycoctonal, establish the presence of a primary hydroxyl group in the alkaloid, and the presence of a methylene next to the nitrogen. The lactam function was unchanged by boiling 6*N* sulphuric acid or by prolonged heating with alkali. The position of the lactam absorption in the infrared (Table II) indicates that the nitrogen is in a six-membered ring.

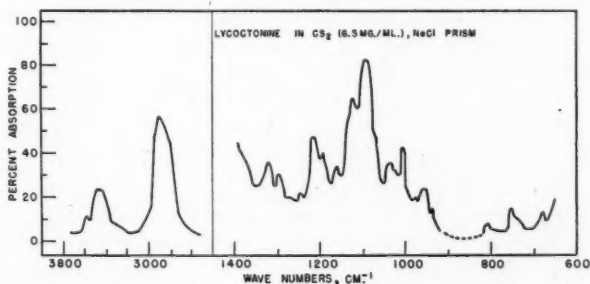
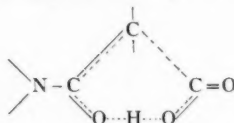


FIG. 2.

The ready decarboxylation of lycoctonamic acid is of great interest. A possible explanation of this fact could be the presence of α - β or β - γ unsaturation. The former however is excluded by the ultraviolet absorption spectrum (only end absorption). Arnold and co-workers (3) and Barton and Brooks (4) have discussed the mechanism of decarboxylation of β - γ unsaturated acids, and the latter workers have shown that the readily decarboxylated triterpene acids are of this

nature. However there is no clear evidence for a double bond in the infrared absorption spectrum of lycoctonine (Fig. 2) or in that of des-(oxymethylene)-lycoctonam, in which the double bond should have shifted were this explanation correct. Lycoctonamic acid was not isomerized to an α - β unsaturated acid with strong alkali (9) and formed no γ -lactone or bromo- γ -lactone on treatment with sulphuric acid (6*N*) or bromine in methanol respectively. The β - γ unsaturated morolic acid fails to give such lactones however (4) and Linstead has shown (22) that the equilibrium between α - β and β - γ unsaturated acids in strong alkali is sometimes very much in favor of the β - γ form.

An alternative explanation of the ready decarboxylation could be that the acid is a malonamic type. Such acids in general decompose below 160°, although cyclohexylmalonamic acid melts at 184° with decomposition. It is possible that the inability of a malonic system to assume the coplanar transition state shown below could raise the decarboxylation temperature markedly.



This could easily be the case in a polycyclic lactam.

In support of the malonamic acid possibility is the p*K* of the acid (4.94 in 50% aqueous methanol) which is nearly the same as that of *N*-(α -carboxybutyryl)-piperidine in the same solvent (5.0). (Acetic acid has a p*K* of 5.58 in 50%

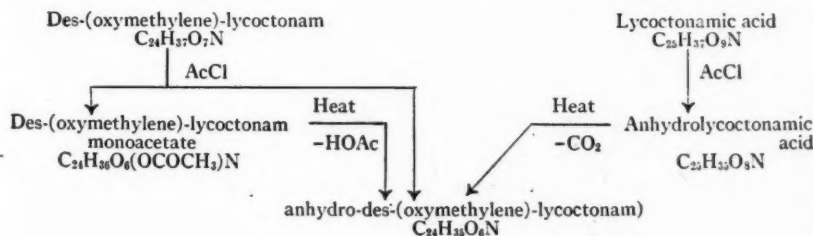
TABLE II
INFRARED SPECTRA
CaF₂ prism, CHCl₃

Compound	Hydroxyls			Carbonyls		
	Primary	Secondary	Tertiary	Lactam	Acid or ester	Aldehyde or ketone
Lycoctonine	3631	3525	3436			
Lycoctonal	—	3537	3445			1725
Hydroxylycoctonine	3644	3515				
Lycoctonam	3601	3525	3451	1613		
Lycoctonam monoacetate		3540	3451	1635	1742	
Lycoctonamic acid		3537	3452	1597	1742	
<i>N</i> -(α -carboxybutyryl)-piperidine				1595	1736	
Methyl lycoctonamate		3525	3445	1641	1720	
<i>N</i> -(α -carbomethoxybutyryl)-piperidine				1634	1736	
Des-(oxymethylene)-lycoctonam		3533	3459	1630		
Des-(oxymethylene)-lycoctonam monoacetate			3470	1633	1744	
Anhydrolycoctonam	Broad			1627		1728
Anhydro-des-(oxymethylene)-lycoctonam				1637		1730
Anhydrolycoctonamic acid				1596	1736	1732

aqueous methanol.) Both acids show the same displacement of the position of the lactam carbonyl peak in the infrared toward lower wave numbers, which disappears when they are esterified (Table II). The shift in pK of -0.2 unit in going from lycoctonine to lycoctonal could also be associated with the proximity of the aldehyde group and the nitrogen.

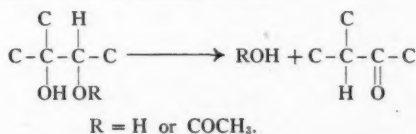
The carboxyl group is most probably attached to a secondary carbon, since it is esterified by methanolic hydrogen chloride, albeit slowly, and the methyl ester is readily saponified. If this be so, then the α -hydrogen should be replaced readily by bromine. Actually the methyl ester reacts very slowly with bromine in carbon tetrachloride, and the experiments have been inconclusive.

Light is shed on the nature of the other two hydroxyls by the reactions outlined below.



The only crystalline compound obtained when lycoctonam was treated with acetyl chloride was anhydrolycoctonam monoacetate. These reactions indicate the presence of a vicinal glycol in the alkaloid, since the dehydration products are ketones. Such dehydrations can involve either hydrogen or carbon migration (2, 32, 33), so it is important to establish whether the hydroxyls are secondary or tertiary. They are inert to Oppenauer oxidation, and are only slowly attacked by chromic acid in glacial acetic acid and by potassium permanganate in acetone. They are untouched by acetic anhydride in pyridine at room temperature. This inertness suggests that they are both tertiary.

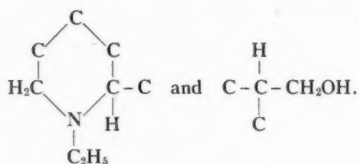
On the other hand, one hydroxyl of des-(oxymethylene)-lycoctonam does acetylate with acetyl chloride, and the resulting acetate is saponified readily by $0.05N$ sodium hydroxide at room temperature. In general, tertiary hydroxyls in polycyclic systems are inert to acetylating agents. A 5- α -hydroxyl in the cholesterol series is an exception, although forcing conditions are required for its acetylation and the 5- α -acetates are only slowly saponified by 2.5% potassium hydroxide solution at room temperature (28, 29). This makes it seem possible that the acetyltable hindered hydroxyl group in lycoctonine is secondary and that the dehydration can be represented thus:



The ready elimination of acetic acid from the acetate on heating is reminiscent of the Slotta-Serini reaction of tertiary-primary and tertiary-secondary vicinal glycol monoacetates (14, 32, 33).

Lycotoctonam consumes 1 mole of periodic acid in around seventy hours, which seems to confirm the hindered vicinal glycol postulate. The product from the reaction will be described shortly.

In the light of the above work the lycotoctonine structure probably contains the following fragments in addition to the glycol illustrated above:



EXPERIMENTAL

For the infrared spectra of the various compounds in the carbonyl and hydroxyl stretching regions see Table II. All infrared absorption spectra were taken on a Perkin-Elmer single beam model 12B spectrophotometer.

Lycotoctonine

The base, after recrystallization from aqueous alcohol, formed colorless needles, m.p. 137° after sintering at 125°.† In 50% aqueous methanol it has a pK of 8.8. The ultraviolet spectrum of the base is recorded in Fig. 1, and its infrared absorption spectrum in carbon disulphide solution in Fig. 2. Its infrared spectrum (determined in chloroform solution with a calcium fluoride prism) showed no absorption in the C = C stretching region. Found for the anhydrous base: active hydrogen (100° in anisole) 0.57, 0.65; C-methyl, 2.33, 2.10; equivalent weight 465. Calcd. for $\text{C}_{25}\text{H}_{41}\text{O}_7\text{N}$: three active hydrogens, 0.65; one C-methyl 3.21; equivalent weight 467.6.

The base consumed no hydrogen in 3*N* hydrochloric acid in the presence of Adams' catalyst, and was recovered unchanged.

Two hundred milligrams of lycotoctonine hydrate was submitted to the conventional Herzig-Meyer N-alkyl determination, and the gases evolved during pyrolysis of the hydriodide were passed into a cold solution of trimethylamine in methanol. The ethanol-soluble salts recovered from the trimethylamine solution yielded 15 mgm. of tiny prisms, m.p. 316° dec. (immersed at 300°) which did not depress the melting point of authentic ethyltrimethylammonium iodide (m.p. 318° dec.). Found: C, 28.58, 28.54; H, 6.30, 6.15. Calcd. for $\text{C}_6\text{H}_{14}\text{NI}$: C, 27.93; H, 6.56.

Lycotoctonine hydriodide, prepared from the base and hydriodic acid, was obtained as colorless needles from acetone m.p. 184° (dec.) when immersed in the bath at 170°. Found: C, 50.49, 50.36; H, 7.18, 7.36%. Calcd. for $\text{C}_{25}\text{H}_{42}\text{O}_7\text{NI}$: C, 50.42; H, 7.11%. This salt proved to be identical with the "methiodide"

† All melting points are corrected to within $\pm 0.5^\circ$.

described earlier by Marion and Manske (25). All attempts to prepare a crystalline methiodide of lycoctonine proved fruitless.

The perchlorate, obtained as usual, separated from alcohol-ether as colorless prisms which crumbled to a powder in air. This melted at 205° (dec.) when immersed in the bath at 180°. Found: C, 53.40, 53.61; H, 7.43, 7.21%. Calcd. for $C_{25}H_{39}O_7N.HClO_4$: C, 53.05; H; 7.12%. Calcd. for $C_{25}H_{41}O_7N.HClO_4$: C, 52.87; H, 7.45%.

The picrate was obtained as needles from acetone-ether, melting at 151-153° (dec.) or 161-163° (dec.). A mixture of the two forms melted at 161-163° (dec.). Found: C, 53.19, 53.31; H, 6.96, 6.68. Calcd. for $C_{25}H_{41}O_7N.C_6H_3O_7N_3$: C, 53.45; H, 6.37. Calcd. for $C_{25}H_{39}O_7N.C_6H_3O_7N_3$: C, 53.59; H, 6.09%.

Hydroxylycoctonine

A suspension of lycoctonine hydrate (1.0 gm.) in 50% aqueous methanol (15 cc.) was stirred with freshly prepared silver oxide (1.5 gm.). The crystalline base soon disappeared and a silver mirror formed. After 90 min. the mixture was filtered and the solid washed with methanol. Fresh silver oxide was added to the filtrate, which proved nearly inert to it, and after one hour the mixture was again filtered. The filtrate was concentrated under reduced pressure to a syrup which was dissolved in dilute sulphuric acid. After extraction with chloroform the acid solution was made alkaline with sodium carbonate and again extracted with chloroform. The 0.948 gm. of base so obtained crystallized readily from ether, giving 678 mgm. of crystals, m.p. 148°, and a further crop of cruder material. The base was dissolved in acetone, the solution concentrated to a syrup, and ether added. After filtration from flocculent colored material the solution was concentrated. Hydroxylycoctonine crystallized as little buttons. After two recrystallizations from ether the base melted at 150-153° when immersed at 130°. $[\alpha]_D^{26} + 12 \pm 1^\circ$ (c, 2.36 in ethanol). It had a pK of 5.8 in 50% aqueous methanol, showed end absorption in the ultraviolet, and no absorption in the carbonyl region in the infrared. Found: C, 62.07, 61.92, 62.24; H, 7.80, 7.94, 8.63%, active hydrogen 0.70, 0.74%; equivalent weight, 488. Calcd. for $C_{25}H_{41}O_8N$: C, 62.09; H, 8.55%; four active hydrogens, 0.83; equivalent weight 483.6. Calcd. for $C_{25}H_{39}O_8N$: C, 62.36; H, 8.16%.

Hydroxylycoctonine formed an hydriodide which crystallized from concentrated methanol solution on addition of acetone, m.p. 194-196° (dec.) when immersed at 175°. Found: C, 49.67, 49.81, 49.67; H, 6.52, 6.82, 6.58%. Calcd. for $C_{25}H_{41}O_8N.HI$: C, 49.11; H, 6.92%. Calcd. for $C_{25}H_{39}O_8N.HI$: C, 49.26; H, 6.61%.

Isolycoctonine

A solution of hydroxylycoctonine (61 mgm.) in ethanol (4 cc.) and concentrated hydrochloric acid (1 cc.) when hydrogenated in the presence of Adams' catalyst (37 mgm.) under 1 atmosphere absorbed 1 mole of hydrogen in 3.5 hr. at room temperature. The catalyst was removed by filtration and the filtrate concentrated to a syrup under reduced pressure. Aqueous ammonia was added to the syrup and the solution extracted with ether. The extract on evaporation yielded 61 mgm. of base which crystallized from a concentrated aqueous solution

as tiny plates, m.p. 94-107°, wt. 60 mgm., $[\alpha]_D$ (anhydrous) + 21 \pm 1° (c, 3.03 in ethanol), pK 6.7 (in 50% aqueous methanol). For the anhydrous base, found: C, 63.87, 64.22, 64.31; H, 8.10, 8.86, 8.56%, equiv. wt. 475. Calcd. for $C_{25}H_{41}O_7N$: C, 64.21; H, 8.84%; equiv. wt. 467.6. Calcd. for $C_{25}H_{39}O_7N$: C, 64.47; H, 8.44%; equiv. wt. 465.6.

Lycotoxonal

To a solution of lycoctonine hydrate (2.0 gm.) in glacial acetic acid (10 cc.) was added 89% pure chromic acid (0.31 gm.). The chromic acid dissolved slowly as the crystals were broken with a glass rod. After three hours the reagent was nearly all reduced but the reaction mixture was allowed to stand a further 20 hr. at room temperature. The acetic acid was then removed by distillation under diminished pressure, the residue dissolved in *N* sulphuric acid, and the solution extracted with chloroform. The combined neutral and acidic material thus extracted weighed 67 mgm. The aqueous liquor was alkalinized with sodium carbonate and extracted with chloroform. On evaporation of the chloroform extract there was obtained 1.85 gm. of base which was dissolved in aqueous methanol. On standing the solution deposited lycotoxonal (0.59 gm.), m.p. 93-103°. The mother-liquor from these crystals deposited a second crop of crystals (0.62 gm.) consisting of lycoctonine. Lycotoxonal was converted to its perchlorate, m.p. 213° (dec.) with loss of solvent at 165° when immersed in the bath at 160°. The nonsolvated salt melted at 211° when placed in the bath at 160°. Found: C, 52.65, 52.40, 52.28; H, 7.07, 6.82, 6.66%. Calcd. for $C_{25}H_{39}O_7N.HClO_4$: C, 53.05; H, 7.12%. Calcd. for $C_{25}H_{37}O_7N.HClO_4$: C, 53.24; H, 6.79%.

The base liberated from the perchlorate crystallized from water as a hydrate, m.p. 93-103° which changed to a form m.p. 116° on standing in air (loss of water?). The pK of the 116° form in 50% aqueous methanol was 8.6; equivalent weight 490 (calcd. for $C_{25}H_{37}O_7N.H_2O$, 483.6). The anhydrous base had $[\alpha]_D^{25}$ + 38 \pm 1° (c, 2.22 in ethanol). The ultraviolet absorption of an ethanol solution of the base had a shoulder near 290 m μ , log ϵ 1.7, due to the carbonyl group. The infrared absorption spectrum of the base dissolved in carbon tetrachloride showed an intense peak at 1725 cm.⁻¹ and a weak band at 2706 cm.⁻¹, both attributable to an aldehyde. Found: C, 64.62, 64.72, 64.36; H, 7.67, 7.93, 7.96%. Calcd. for $C_{25}H_{37}O_7N$: C, 64.76; H, 8.04%. Calcd. for $C_{25}H_{39}O_7N$: C, 64.47; H, 8.44%.

Oxidation of Lycotoxonal

To a solution of hydrated lycotoxonal (50 mgm.) in pure acetone (5 cc.) containing water (0.1 cc.) and acetic acid (0.1 cc.), powdered potassium permanganate (56 mgm.) was added in small quantities over a period of one hour, at room temperature. The permanganate was reduced rapidly at first, but the last 6 mgm. persisted for more than one hour. The excess permanganate and the manganese dioxide were reduced with sulphur dioxide and the white inorganic precipitate was filtered and washed with acetone. The combined filtrate and washings were evaporated to dryness under diminished pressure, the residue dissolved in water and separated into acid and neutral fractions. There was thus obtained 6 mgm. of an acid which separated from water in the form of needles (4 mgm.), m.p. 198° (dec.) when immersed at 160°. In admixture with lycoctonamic acid,

m.p. 199° (dec.). The neutral fraction (30 mgm.) crystallized from ether, giving 10 mgm., m.p. 177°, which after recrystallization from acetone-ether consisted of prisms, m.p. 179-182° (imm. at 140°). In admixture with des-(oxymethylene)-lycoctonam this melted at 181°.

Reduction of Lycoctonal

A solution of lycoctonal hydrate (20 mgm.) and sodium boron hydride (30 mgm.) in 50% aqueous methanol (2 cc.) was warmed for a few minutes on the steam bath and allowed to stand for 30 min. at room temperature. The excess sodium boron hydride was decomposed with acetic acid, the solution alkalinized with potassium hydroxide and extracted with methylene dichloride. The extract on evaporation yielded 18 mgm. of a product which crystallized from water as colorless needles softening at 126° and melting at 134° (dec.). In admixture with lycoctonine the melting point was undepressed. The base formed a picrate, m.p. 163° either alone or after mixing with lycoctonine picrate.

Desoxylycoctonine

To a solution of lycoctonal hydrate (47 mgm.) in triethylene glycol (1 cc.) (15) were added 85% hydrazine hydrate (0.5 cc.) and potassium hydroxide (80 mgm.), and the mixture was refluxed for 45 min. The condenser water was removed and the bath temperature gradually raised to 195°. Water was allowed again to flow through the condenser and the residual solution refluxed for four hours. The solution was cooled and water (5 cc.) together with potassium hydroxide (0.2 gm.) was added. A crop of glistening plates separated, wt. 33 mgm., m.p. 81.5-84.5. An ether extract of the mother-liquor gave an oil which when dissolved in water (0.5 cc.) yielded 6 mgm. of plates, m.p. 76.5°. The combined product separated from aqueous methanol as small colorless plates, m.p. 74.5-76.5°. The two melting points (84.5 and 76.5°) seem to correspond to two different crystalline forms. $[\alpha]_D^{24}$ (anhydrous) + 50 ± 2° (c, 1.85 in ethanol). The compound showed only end absorption in the ultraviolet. Found: C, 66.88; H, 8.90; N, 3.64%. Calcd. for $C_{25}H_{41}O_6N$: C, 66.49; H, 9.15; N, 3.10%. Calcd. for $C_{25}H_{39}O_6N$: C, 66.79; H, 8.74; N, 3.12%.

Desoxylycoctonam

To a solution of desoxylycoctonine (170 mgm.) in acetone (15 cc.) containing one drop of acetic acid and one drop of water was added potassium permanganate in 15 mgm. portions. At first the oxidation was rapid, but it slowed gradually. In 3.5 hr. 100 mgm. of oxidant was used up. Another 15 mgm. persisted for 1.5 hr. The excess potassium permanganate was reduced with sodium bisulphite after the addition of 2 cc. of water. The mixture was filtered, the insoluble material washed with acetone and methanol, and the combined filtrate and washings evaporated to dryness. The residue was dissolved in chloroform and the solution washed with aqueous alkali and with acid. It was thus separated into 4 mgm. of acid, 24 mgm. of base, and 121 mgm. of neutral material. The neutral fraction was crystallized from ether (88 mgm.). After two recrystallizations from a very concentrated acetone solution by the addition of ether, it melted at 160-168°. When chromatographed on 1.5 gm. of alumina, activity 3-4 (Brockmann scale), 80 mgm. of this product yielded 70 mgm. of material readily eluted with chloro-

form. This main fraction of the chromatogram when crystallized from ether gave 45 mgm. of desoxylycoctonam, m.p. 162-169°. Twice recrystallized from ether it melted at 167° after softening at 161°. $[\alpha]_D^{28} + 58 \pm 1^\circ$ ($c = 2.10$ in ethanol). Found: C, 64.73, 64.68; H, 8.54, 8.68%. Calcd. for $C_{25}H_{39}O_7N$: C, 64.47; H, 8.44%. Calcd. for $C_{25}H_{37}O_7N$: C, 64.76; H, 8.04%.

Oxidation of Lycoctonine

To hydrated lycoctonine (10 gm.) dissolved in acetone (200 cc.) containing acetic acid (5 cc.) and water (5 cc.), powdered potassium permanganate (7 gm.) was added portionwise over a period of 2.5 hr. with agitation after each addition. The last portion (0.25 gm.) persisted for 45 min. Sulphur dioxide was bubbled into the reaction mixture until the manganese dioxide had been converted to sulphate. The solution was then filtered and the filtrate concentrated to a syrup under reduced pressure. (Alternatively, the manganese dioxide was filtered off and washed by suspension first in acetone and then in aqueous sodium carbonate. After concentration of the acetone solutions the sodium carbonate washings were added to the residue and the solution acidified.) The syrup was taken up in water, 6*N* sulphuric acid (5 cc.) added, and the combined neutral and acidic material extracted with chloroform. The aqueous layer was alkalized with sodium carbonate and extracted with chloroform; this extract on evaporation yielded 0.81 gm. of amorphous base. The chloroform extract containing the acidic and neutral material was shaken with aqueous sodium carbonate. The chloroform solution retained the neutral product (8.5 gm.) which was obtained on evaporation of the solvent. The sodium carbonate solution on acidification and extraction yielded the acid fraction (0.40 gm.) which on crystallization from water (1 cc.) gave lycoctonamic acid, m.p. 202°, wt. 0.27 gm. (see below).

The neutral fraction was separated by chromatography in benzene solution on 150 gm. of alumina (acid washed; activity 3-4, Brockmann scale). Elution of the chromatogram gave 37 fractions each consisting of 50 cc. of eluate.

TABLE III
ELUATES FROM CHROMATOGRAM

Fractions	Eluting solvent	Wt. in gm.
1-4	Benzene	Trace (amorphous)
5,6	Chloroform	1.70
7	"	0.37
8-20	"	1.56
21-25	Chloroform + 1% methanol	0.26
26-34	Chloroform + 2% methanol	4.40
35-37	Methanol	0.47
	Total	8.76

Fractions 5 and 6 crystallized when taken up in ether, yielding 1 gm. of des-(oxymethylene)-lycoctonam m.p. 185-188° (see below). Fractions 7-34, as grouped in Table III, were converted to acetates by treating with acetic anhydride and pyridine for 20 hr. at room temperature. The products all crystallized from ether

yielding a total of 5.6 gm. of lycoctonam monoacetate. It has not so far been possible to induce crystallization of the remaining fractions.

Lycoctonam monoacetate crystallized from a very concentrated acetone solution on addition of ether as colorless prismatic needles, m.p. 189° after softening at 182° (imm. at 150°). $[\alpha]_D^{24} + 57.5 \pm 1^{\circ}$ (*c*, 2.45 in ethanol). Found: C, 62.34, 62.13; H, 7.73, 7.64; N, 2.69, 2.64; OCH_3 , 19.36, 19.42%. Calcd. for $\text{C}_{27}\text{H}_{41}\text{O}_9\text{N}$: C, 61.93; H, 7.89; N, 2.68; 4OCH_3 , 23.70%. Calcd. for $\text{C}_{27}\text{H}_{39}\text{O}_9\text{N}$: C, 62.17; H, 7.54; N, 2.69; 4OCH_3 , 23.79%.

The monoacetate was rapidly saponified by 5% potassium hydroxide in aqueous methanol without the lactam function being affected. The solution was evaporated under reduced pressure to a syrup, water was added, and the product extracted by shaking with chloroform. Lycoctonam, obtained by evaporation of the chloroform extract, crystallized readily from a concentrated aqueous solution as a dihydrate in the form of needles, m.p. 95° after softening at 88° . The anhydrous lactam had $[\alpha]_D^{24} + 66.4 \pm 1^{\circ}$ (*c*, 2.59 in ethanol). Found (hydrate): C, 58.10, 57.95; H, 8.51, 8.33; N, 2.83, 2.75; OCH_3 , 19.13, 18.89%. Calcd. for $\text{C}_{28}\text{H}_{39}\text{O}_8\text{N} \cdot 2\text{H}_2\text{O}$: C, 58.01; H, 8.37; N, 2.71; 4OCH_3 , 23.97; 3OCH_3 , 17.99%. Found (anhydrous): C, 62.56, 62.37; H, 7.91, 7.87%. Calcd. for $\text{C}_{28}\text{H}_{39}\text{O}_8\text{N}$: C, 62.36; H, 8.16%. Calcd. for $\text{C}_{28}\text{H}_{37}\text{O}_8\text{N}$: C, 62.61; H, 7.78%. The ultraviolet spectrum of lycoctonam shows end absorption only. The infrared absorption spectrum in carbon disulphide solution had peaks at 757.5, 801, 909, 977, 1020, 1040, 1094, 1127, 1153, 1176, 1203, 1252, 1296, 1313, and 1347 cm^{-1} . Lycoctonam steadily reduced periodic acid at pH 5 until after 72 hr. the reaction stopped, when the total consumption was equivalent to 1 mole of the reagent. The product of this reaction will be described in a forthcoming paper.

Reduction of Lycoctonam Acetate

To a solution of the monoacetate (81 mgm.) in pure dioxane (5 cc.) was added a 10% solution of lithium aluminum hydride in ether (3 cc.). The mixture was refluxed in a slow stream of nitrogen until the ether had evaporated (15 min.) and then refluxed a further 15 min. The excess reagent was decomposed with methanol, water was added, and the precipitate of aluminum hydroxide filtered with suction and washed by suspension first in acetone and then in methanol. The product of the reaction recovered from the filtrate and washings were separated into a trace of neutral material and 60 mgm. of base which crystallized from water as a hydrate, m.p. $119\text{--}126^{\circ}$ (wt. 40 mgm.). In admixture with lycoctonine, it melted at $128\text{--}135^{\circ}$. Half the base was converted to a picrate, m.p. $145\text{--}148^{\circ}$, which in admixture with lycoctonine picrate melted at $147\text{--}150^{\circ}$. The remainder of the base was converted to a perchlorate, m.p. $203\text{--}204^{\circ}$ (dec.) which after mixing with lycoctonine perchlorate had m.p. 205° (dec.).

Oxidation of Lycoctonam

Powdered potassium permanganate in portions of 0.5 gm. was added to a solution of lycoctonam hydrate (3.8 gm.) in acetone (90 cc.) containing water (5 cc.) and acetic acid (5 cc.). The first 0.5 gm. of potassium permanganate was reduced in one hour at 40° . A further 3 gm. was added in similar portions over a period of six hours. A further 0.8 gm. was reduced overnight at room temperature.

The product was worked up as described for the oxidation of lycoctonine (sulphur dioxide method) and separated into acid and neutral fractions. The acid fraction (1.56 gm.) crystallized when dissolved in water. It consisted of lycoctonamic acid, wt. 1.3 gm. The neutral fraction (1.7 gm.) was acetylated and the crude acetate (1.9 gm.) yielded 1.0 gm. of crystalline lycoctonam monoacetate, m.p. 188° either alone or in admixture with an authentic sample.

Anhydrolycoctonam Acetate

Lycoctonam monoacetate (0.605 gm.) was refluxed with acetyl chloride (10 cc.) in a nitrogen atmosphere for five hours. The neutral product of the reaction weighed 0.601 gm. It was dissolved in ether and the solution deposited colorless needles, m.p. 189-196°, wt. 0.417 gm. It was recrystallized three times from a concentrated acetone solution by the addition of ether, when it melted at 199° after softening at 191°. $[\alpha]_D^{25} + 7 \pm 2^\circ$ (c, 1.135 in ethanol). Found: C, 63.96, 63.82; H, 7.55, 7.43; OCH₃, 24.86%. Calcd. for C₂₇H₃₉O₈N: C, 64.13; H, 7.78; 4OCH₃, 24.55%. Calcd. for C₂₇H₃₇O₈N: C, 64.39; H, 7.41; 4OCH₃, 24.64%.

Anhydrolycoctonam

A solution of anhydrolycoctonam monoacetate (346 mgm.) in methanol (5 cc.) and water (1 cc.) containing potassium hydroxide (0.4 gm.) was allowed to stand at room temperature for 90 min., and heated on the steam bath for 15 min. The solution was concentrated under reduced pressure, diluted with water, acidified, and extracted with chloroform. Evaporation of the extract left a residue (328 mgm.) which was dissolved in ether. This solution deposited anhydrolycoctonam (298 mgm.) as glistening needles, m.p. 148-150°, which after recrystallization from acetone-ether melted at 150-151°. $[\alpha]_D^{24} + 24 \pm 1^\circ$ (c, 3.25 in ethanol). Found: C, 65.21, 65.39, 65.00; H, 7.98, 7.78, 8.05; N, 3.54, 3.82, 3.69; OCH₃, 24.95%. Calcd. for C₂₅H₃₇O₇N: C, 64.77; H, 8.05; N, 3.02; 4OCH₃, 26.78%. Calcd. for C₂₅H₃₅O₇N: C, 65.06; H, 7.64; N, 3.04; 4OCH₃, 26.88%. The compound in ethanolic hydrochloric acid was inert to hydrogenation over Adams' catalyst at room temperature and a pressure of 1 atmosphere. The ultra-violet spectrum in 95% ethanol showed λ_{\min} 265 m μ , log ϵ 1.20 and λ_{\max} 305 m μ , log ϵ 1.89.

Lycoctonamic Acid

This acid was obtained as described under oxidation of lycoctonam. It crystallized as a polyhydrate in colorless needles either from a concentrated methanol solution on dilution with water, or from a solution in boiling water on cooling. Part of the water of hydration was lost on standing in air with, however, retention of crystal form. The initial hydrate lost water vigorously when immersed in a bath at 170°; it then resolidified and melted at 204° (dec.). Both the less hydrated and the anhydrous acid show only the final melting point. $[\alpha]_D^{26}$ (anhydrous) $+ 99 \pm 2^\circ$ (c, 2.19 in ethanol); pK in 50% aqueous methanol 4.94. Found (anhydrous): C, 60.52, 60.54, 60.64; H, 7.14, 7.57, 7.84%; equivalent weight 508, 506. Calcd. for C₂₅H₃₇O₉N: C, 60.60; H, 7.53%; equivalent weight, 495.6. Calcd. for C₂₅H₃₅O₉N: C, 60.84; H, 7.15%; equiv. wt. 493.54. The acid was unchanged by 10 min. refluxing in pyridine or 12 hr. on the steam bath in

16% potassium hydroxide solution. The acid did not react appreciably with bromine in methanol in 17 hr. at room temperature.

Methyl Lycoctonamate

A solution of anhydrous lycoctonamic acid (25 mgm.) in 2% hydrogen chloride in methanol (5 cc.) was allowed to stand at room temperature for 20 hr. From this solution 18 mgm. of acid was recovered unchanged together with 5 mgm. of the methyl ester, m.p. 159-163°. However, treatment of a methanol solution of the acid with excess ethereal diazomethane gave a quantitative yield of the ester which separated from ether as needles, m.p. 161-166°. After chromatographing on alumina and recrystallizing twice from ether it melted at 163-167°. $[\alpha]_D^{24} + 64 \pm 2^\circ$ ($c = 2.09$ in ethanol). Found: C, 61.62; H, 6.95; N, 3.20%. Calcd. for $C_{26}H_{37}O_9N$: C, 61.53; H, 7.35; N, 2.76%. Calcd. for $C_{26}H_{35}O_9N$: C, 61.28; H, 7.71; N, 2.75%.

A quantity of the ester (30 mgm.) was hydrolyzed by refluxing with methanol (2 cc.) and 0.1N potassium hydroxide (3 cc.) for 30 min. It yielded 28 mgm. of acid which crystallized from water as needles, wt. 22 mgm. m.p. 197° (dec.). In admixture with lycoctonamic acid, m.p. 199° (dec.).

Reduction of Methyl Lycoctonamate

To a solution of the ester (80 mgm.) in dioxane (8 cc.) was added an approximately 5% solution of lithium aluminum hydride in ether (9 cc.). The reduction was carried out as described for lycoctonam acetate. A base was obtained (67 mgm.) which crystallized from water as needles, wt. 40 mgm., m.p. 120-135° (dec.) either alone or when mixed with lycoctonine. The base was converted to the perchlorate, m.p. 205° (dec.), and also to the picrate, m.p. 163° (dec.). These melting points were unaltered when the salts were mixed respectively with the corresponding salts of lycoctonine.

Anhydrolycoctonamic Acid

To highly hydrated lycoctonamic acid (164 mgm.) and two drops of water, acetyl chloride (3 cc.) was added. Hydrogen chloride was evolved. The solution was refluxed in an atmosphere of carbon dioxide for three hours and then concentrated to a syrup under reduced pressure. Water (5 cc.) together with potassium hydroxide (0.3 gm.) was added and the solution heated on the steam bath for 30 min. The solution was acidified and extracted with chloroform. Evaporation of the extract left a residue (139 mgm.) which crystallized from a concentrated methanol solution, wt. 99 mgm., m.p. 177-178° (dec.). After two recrystallizations from methanol the colorless prisms melted at 178-179° (dec.) when immersed at 155°. $[\alpha]_D^{24} + 54 \pm 2^\circ$ ($c = 2.36$ in chloroform). Found: C, 63.19, 63.09; H, 7.69, 7.54%. Calcd. for $C_{25}H_{38}O_8N$: C, 62.87; H, 7.39%. Calcd. for $C_{25}H_{36}O_8N$: C, 63.14; H, 6.99%.

Decarboxylation of Anhydrolycoctonamic Acid

The acid (23 mgm.) was heated at 185° for five minutes in an atmosphere of carbon dioxide. The product, after distillation at 180° (bath temperature) under 0.2 mm. pressure, consisted of a colorless crystalline solid. After recrystallization from acetone-ether, it weighed 17 mgm. and melted at 196° after softening at

187°. In admixture with anhydro-des-(oxymethylene)-lycoctonam, m.p. 198° after softening at 189°.

Des-(oxymethylene)-lycoctonam

Anhydrous lycoctonamic acid (0.532 gm.) was introduced in a flask connected to a trap cooled with dry-ice-acetone which in turn was connected to a bubbler containing aqueous barium hydroxide. A slow stream of nitrogen was kept flowing through the apparatus while the flask was heated to 205-210° and kept at that temperature for 7 min. The decarboxylation was essentially complete in 3 min. The reaction vessel was swept with nitrogen as it cooled (30 min.). Only a trace of volatile material other than carbon dioxide was formed. The barium carbonate precipitate removed from the second trap weighed 0.216 gm. (84% of theoretical for 1 mole of carbon dioxide). The decarboxylated product weighed 0.487 gm. (theory, 0.488 gm.); it crystallized readily from ether as prisms, m.p. 179°. After recrystallization from acetone-ether, m.p. 184° with softening at 182°. This substance proved identical (mixed melting point and comparison of infrared absorption spectra) with the product of the oxidation of lycoctonine with potassium permanganate. Chromatography of this substance on 30 times its weight of acid-washed alumina of activity 4 (Brockmann scale) from which it was eluted slowly as the solvent was changed gradually from benzene to chloroform to 1% methanol in chloroform, raised the melting point to 185-188° (imm. at 160°). $[\alpha]_D^{26} + 49 \pm 1^\circ$ ($c = 1.87$ in ethanol). A form melting at 149° was frequently obtained from acetone-ether. A mixture of this with the 188° form melted at 188°. The compound showed only end absorption in the ultra-violet region. The infrared absorption spectrum in carbon disulphide solution contained absorption peaks at 681, 727, 801, 903, 929, 966, 993, 1009, 1033, 1041, 1097, 1126, 1155, 1175, 1193, 1206, 1219, 1252, 1270, 1292, 1347 cm^{-1} . Found: C, 64.39, 64.14, 63.96; H, 8.43, 8.61, 8.60; N, 4.28, 4.19%. Calcd. for $\text{C}_{24}\text{H}_{37}\text{O}_7\text{N}$: C, 63.83; H, 8.26; N, 3.10%. Calcd. for $\text{C}_{24}\text{H}_{38}\text{O}_7\text{N}$: C, 64.13; H, 7.85; N, 3.12%. The compound in solution in ethanolic hydrochloric acid was inert to hydrogen under a pressure of 1 atmosphere in the presence of Adams' catalyst.

Des-(oxymethylene)-lycoctonine

To a solution of des-(oxymethylene)-lycoctonam (200 mgm.) in dioxane (9 cc.) was added approximately 1.2×10^{-3} mole of lithium aluminum hydride in ether (10 cc.). The reduction and isolation of the crude product were carried out as described for lycoctonam acetate. The product was taken up in dilute acid and the solution extracted with chloroform to remove a trace of neutral material. After addition of excess sodium carbonate the base was extracted with ether and the extract evaporated to remove the solvent. An oily base (186 mgm.) was left which yielded 202 mgm. of crystalline perchlorate, m.p. 146° (dec.) after sintering at 136°. Found: C, 54.00, 53.84; H, 7.36, 7.35%. Calcd. for $\text{C}_{24}\text{H}_{37}\text{O}_6\text{N} \cdot \text{HClO}_4$: C, 53.78; H, 7.15%. The base recovered from the perchlorate distilled readily at 160° at 10^{-5} mm. $[\alpha]_D^{25} + 27.8 \pm 0.5^\circ$ ($c = 2.19$ in ethanol); pK 8.9 in aqueous methanol, equivalent weight by titration 448 (calcd. 437.6). Found: C, 66.20; H, 8.68%. Calcd. for $\text{C}_{24}\text{H}_{37}\text{O}_6\text{N}$: C, 66.18; H, 8.56%. Calcd. for $\text{C}_{24}\text{H}_{39}\text{O}_6\text{N}$: C, 65.87; H, 8.98%.

An attempt to prepare the methiodide by heating with methyl iodide and methanol at 100° in a sealed tube resulted in the formation of the hydriodide, identical with that prepared from the base and hydriodic acid m.p. 173-174° (dec.) (imm. at 140°). Found: C, 50.29, 50.08; H, 7.09, 7.16%. Calcd. for $C_{24}H_{37}O_6N.HI$: C, 51.16; H, 6.80%. Calcd. for $C_{24}H_{39}O_6N.HI$: C, 51.97; H, 7.13%.

Des-(oxymethylene)-lycoctonam Monoacetate

Des-(oxymethylene)-lycoctonam (200 mgm.) was refluxed with acetyl chloride (4.5 cc.) for five hours. The excess reagent was distilled under reduced pressure, the residual product dissolved in chloroform, and the solution washed with sodium carbonate solution. The chloroform solution on evaporation left a residue (230 mgm.) which crystallized from ether in four crops: crop 1, 23 mgm.; crops 2 and 3, 78 mgm.; crop 4, 30 mgm. (For crops 2 and 3 see below.) Crops 1 and 4 were combined and recrystallized three times by addition of ether to a very concentrated acetone solution. The monoacetate thus obtained melted at 231-234° (dec.) (imm. at 210°). $[\alpha]_D^{25} + 1 \pm 1^\circ$ ($c = 1.82$ in ethanol). Found: C, 63.18; H, 7.95; OCH_3 , 26.78%. Calcd. for $C_{26}H_{39}O_8N$: C, 63.29; H, 7.97; $4OCH_3$, 25.12%. Calcd. for $C_{26}H_{37}O_8N$: C, 63.52; H, 7.59%. The compound showed only end absorption in the ultraviolet. The infrared absorption spectrum contained a peak at 1237 cm^{-1} , characteristic of an O-acetyl group.

The acetate (21 mgm.) in 4 cc. of 0.05*N* sodium hydroxide in 50% aqueous methanol was allowed to stand at room temperature for four hours. The neutral product crystallized from ether, giving a first crop of 15 mgm. m.p. 183°, mixed m.p. with des-(oxymethylene)-lycoctonam 184°. A second crop of 3 mgm. melted at 152°, mixed m.p. with des-(oxymethylene)-lycoctonam 183°.

Anhydro-des-(oxymethylene)-lycoctonam

Crops 2 and 3 obtained from the acetylation of des-(oxymethylene)-lycoctonam were combined and recrystallized from acetone-ether. The product (68 mgm.) melted at 192-197°. After four recrystallizations and chromatography on alumina, it melted at 196-199°. $[\alpha]_D^{25} - 7 \pm 1^\circ$ ($c = 1.59$ in ethanol). Found: C, 66.70; H, 8.30; OCH_3 , 25.42%. Calcd. for $C_{24}H_{35}O_6N$: C, 66.50; H, 8.14; $4OCH_3$, 28.62%. Calcd. for $C_{24}H_{33}O_6N$: C, 66.80; H, 7.71; $4OCH_3$, 28.76%. The ultraviolet spectrum in 95% ethanol showed a maximum at 305 μ , $\log \epsilon$ 1.92.

Pyrolysis of Des-(oxymethylene)-lycoctonam Monoacetate

Samples of the monoacetate heated above the melting point evolved acetic acid as judged by the odor and the fact that Congo red paper was turned blue. The monoacetate (66 mgm.) was heated at 235-240° for 10 min. in a water pump vacuum. The product was distilled in an air-bath at 200° at 0.5 mm. The colorless distillate (52 mgm.) was crystallized from ether, m.p. 192°. After three recrystallizations from ether it melted at 189-197° (imm. at 172°), wt. 27 mgm. In admixture with anhydro-des-(oxymethylene)-lycoctonam, m.p. 187-198°. The product, once more recrystallized, had an infrared absorption spectrum exactly superposable on that of anhydro-des-(oxymethylene)-lycoctonam.

Chromic Acid Oxidation of Des-(oxymethylene)-lycoctonam

A solution of 135 mgm. of the lactam and 27 mgm. of chromium trioxide in 5 cc. of acetic acid was left overnight at room temperature, during which time the reagent was all reduced. The 136 mgm. of neutral product was separated by

chromatography on alumina (acid washed, activity 3-4). Benzene-chloroform mixtures and chloroform eluted 78 mgm. which crystallized in part from ether giving 5 mgm. of prismatic needles m.p. 138°. This was combined with the same product from a similar run. Two recrystallizations raised the melting point to 160°. The infrared absorption spectrum of this compound in chloroform had peaks at 1759, 1706, and 1639 cm^{-1} attributable to lactone, ketone, and lactam carbonyl respectively. Methanol eluted a further 76 mgm. which was predominantly des-(oxymethylene)-lycoctonam.

Attempted Oppenauer Oxidation of Des-(oxymethylene)-lycoctonam

Des-(oxymethylene)-lycoctonam was recovered unchanged after two hours refluxing with benzoquinone and aluminum tert-butoxide in benzene or after 0.5 hr. refluxing with the same reagents in toluene. The same results were obtained with a 50% benzene-cyclohexanone mixture or with a 7:5 by volume mixture of acetone-toluene, all with aluminum tert-butoxide as catalyst. Use of the modified Oppenauer oxidation using potassium tert-butoxide and fluorenone in benzene proved equally fruitless.

N-(α -Carboxybutyryl)-piperidine

Ethyl ethylmalonate (20 gm.) containing piperidine (3 gm.) was refluxed for 90 min. under an air condenser through which the ethanol formed could evaporate. The bulk of the ethyl ethylmalonate was distilled under 15 mm. pressure and the residual product under 0.5 mm. (bath temperature 130°), the forerun of ethyl ethylmalonate being discarded. The mobile oil obtained was hydrolyzed at room temperature with potassium hydroxide in 50% aqueous methanol. The basic solution was extracted with ether, then acidified and extracted with methylene dichloride. Evaporation of the methylene dichloride extract left a residue which after two crystallizations from ether-petroleum ether melted at 74°, wt. 2.2 gm. After three further recrystallizations the melting point was 73.5-74°. pK 5.0 in 50% aqueous methanol; pK 3.86 in water. Found: C, 60.42, 60.30; H, 8.37, 8.05; N, 7.07%. Neutral equivalent, 201.201. Calcd. for $\text{C}_{10}\text{H}_{17}\text{O}_3\text{N}$: C, 60.27; H, 8.60; N, 7.03; neutral equivalent 199.25. The methyl ester of the acid, prepared with the aid of diazomethane, was a colorless oil distilling at approximately 90°, 0.2 mm.

Pseudoaconine Tetraacetate

The ultraviolet spectrum of a sample of this substance described previously (26) showed no maximum and no absorption beyond 260 $\text{m}\mu$. In the infrared absorption spectrum, in the 1600-1800 cm^{-1} region only one absorption peak is present which is attributable to the acetate groups.

Pyropseudoaconine Triacetate

The ultraviolet spectrum of a sample described previously (26) has λ_{min} 223 $\text{m}\mu$, $\log \epsilon$ 3.54; λ_{max} 246 $\text{m}\mu$, $\log \epsilon$ 3.78 in 95% ethanol. In the infrared absorption spectrum (CHCl_3 , CaF_2) a weak band is present at 1634 cm^{-1} in addition to the acetate peak.

Aconitine Hydriodide

Recrystallized Merck aconitine, m.p. 200° (dec.) was converted to the hydriodide, m.p. 224° (dec.) (imm. at 210°). The ultraviolet absorption spectrum of the

salt in 95% ethanol showed λ_{\max} 225 m μ , log ϵ 4.34; λ_{\min} 260 m μ , log ϵ 2.83; λ_{\max} 274 m μ , log ϵ 2.97.

Pyraconitine Salts

Crystalline aconitine, m.p. 200° (dec.) (0.40 gm.), was heated at 196–200° for five minutes in a metal bath. After cooling, the product was dissolved in chloroform, the solution washed with aqueous sodium carbonate, dried, decolorized with a little acid-washed activated alumina, and evaporated. A frothy basic residue was left (0.37 gm.) which could not be crystallized. It was converted to the hydriodide (303 mgm.) which after three recrystallizations from methanol–acetone melted at 274° (dec.) (imm. at 250°). Pyraconitine hydriodide has recorded m.p. 220.5° (8). The ultraviolet absorption spectrum in 95% ethanol showed λ_{\max} 225 m μ , log ϵ 4.36; λ_{\min} 260 m μ , log ϵ 2.90; λ_{\max} 274 m μ , log ϵ 2.98.

The hydrochloride prepared from the base recovered from the hydriodide melted at 248° (dec.) (imm. at 230°). The literature records m.p. 249° (dec.) (8).

ACKNOWLEDGMENT

The authors wish to thank Dr. R. A. McIvor for his help in the search of the recent Russian literature, and Dr. R. N. Jones and Mr. R. Lauzon for taking the infrared absorption spectra.

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DISILYL ALKANES¹By W. D. ENGLISH,² A. TAURINS, AND R. V. V. NICHOLLS

ABSTRACT

By passing appropriate dichloroalkanes, e.g. 1,1-dichloroethane, over a mixture of silicon and copper at elevated temperature, the new compounds 1,1-bis-trichlorosilylethane, 1,2-bis-trichlorosilylpropane, and 1,4-bis-trichlorosilylbutane and the previously reported bis-trichlorosilylmethane, dichlorosilyltrichlorosilylmethane, and 1,2-bis-trichlorosilylethane were synthesized. Trichloromethane yielded perchlorodisilane; 2-chloropropane gave propene.

By causing the chlorosilylalkanes to react with lithium aluminum hydride, disilylmethane, 1,1-disilylethane, 1,2-disilylethane, and 1,2-disilylpropane were synthesized.

Several physical constants of the compounds were measured.

INTRODUCTION

Few organo-silicon compounds have been prepared which contain more than one silicon atom with each silicon having only one silicon-carbon bond. Of compounds with the structure $(\text{SiX}_3)_2\text{R}$ where X is halogen, only those where R represents methylene (11,18), dimethylene (3,11), and trimethylene (3) have been described, while no compound of the type $(\text{SiH}_3)_2\text{R}$ has been reported.

The Rochow synthesis of organo-silicon compounds (15,16,17) extended to the use of polyhaloalkanes by Patnode and Schiessler (11,12) provides a simple method for the introduction of two silicon atoms into a molecule. The process developed by Finholt *et al.* (5,6) using lithium aluminum hydride is suitable for the replacement of chlorine with hydrogen.

In this investigation dichloromethane, 1,1- and 1,2-dichloroethane, 1,2-dichloropropane, and 1,4-dichlorobutane were used as sources of the corresponding bis-trichlorosilylalkanes. Trichloromethane did not form tris-trichlorosilylmethane, nor did 2-chloropropane give 2-trichlorosilylpropane. Reduction of the bis-trichlorosilyl derivatives of methane, ethane, and propane yielded the corresponding disilylalkanes.

EXPERIMENTAL³

The silicon used in this research contained iron, 0.89%; aluminum, 0.78%; calcium, 0.2%; manganese, 0.03%. The various haloalkanes were used as received. Cylinder nitrogen was deoxygenated in the manner described by Uhrig, Roberts, and Levin (21).

Reaction tubes of stainless steel or Pyrex were employed. They were heated in electric furnaces controlled by autotransformers.

The condensing system consisted of three sections. The first was a trap maintained above the boiling point of the reactant but below that of the product. Then a condenser and flask kept at 0°C., and finally a trap cooled by Dry Ice. Uncondensed gases passed out through a calcium chloride drying tube.

Vapor pressures were measured dynamically in an apparatus similar to that

¹ Manuscript received February 6, 1952.

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³ Melting and boiling points are corrected.

TABLE I
 THE ROCHOW REACTION

Reactant	Flow rate, gm./hr.	Furnace temperature, °C.	Product	Yield, %	% Chlorine		% Silicon		Mol. wt.	
					Calc.	Found	Calc.	Found	Calc.	Found
Dichloromethane	21	350-390	$\begin{cases} \text{Cl}_3\text{SiCH}_2\text{SiCl}_3 \\ \text{Cl}_2\text{HSiCH}_2\text{SiCl}_3 \end{cases}$	8 3	75.2 71.2	75.8, 75.1 74.7, 74.8 71.3, 70.3 69.7	18.8 22.5	18.7 20.0	283 249	291 260
1,1-Dichloroethane	30	360-380	$\text{Cl}_3\text{SiCH}(\text{CH}_3)\text{SiCl}_3$	8	71.7	71.8, 70.3 70.2, 71.1	18.9	18.7, 19.0 18.9	-	-
1,2-Dichloroethane	120	> 330	$\text{Cl}_3\text{SiCH}_2\text{CH}_2\text{SiCl}_3$	1	71.7	71.8	18.9	19.0	297	299
1,2-Dichloropropane	107	300-320 (\nearrow 360)	$\text{Cl}_3\text{SiCH}_2\text{CH}(\text{CH}_3)\text{SiCl}_3$	1.6	68.5	68.5, 67.2 67.0	18.1	18.5, 18.0 19.2	-	-
1,4-Dichlorobutane	73	330-360	$\text{Cl}_3\text{Si}(\text{CH}_2)_3\text{SiCl}_3$	0.01	65.6	65.5	17.2	17.2	-	-
Trichloromethane	83	340-360	$\text{Cl}_3\text{SiSiCl}_3$	0.3	-	-	-	-	-	-
2-Chloropropane	45	360-410	$\text{CH}_3\text{CH}=\text{CH}_2$	12	Identified as dibromide					

described by Mack and France (10). Densities were determined with a Fisher-Davidson gravitometer. Molecular weights were determined cryoscopically in benzene.

Refractive indices were measured with an Abbé refractometer. Although precautions were taken, after only 10 measurements with *bis*-trichlorosilylmethane, enough polymerization had taken place on the prisms to render the instrument useless. The prisms had to be reground. Dr. M. C. Hunter advised us (8) that no compound containing more than three silicon-halogen bonds should be placed in a prism refractometer.

A mixture was made of silicon and copper powders, 9:1 by weight, both powders - 50, + 100 mesh. Enough powder was placed in the reaction tube to half fill it. Nitrogen was passed through the tube while the temperature was raised. Then the alkyl halide was added at such a rate that its vapor formed a 1:1 mixture with the nitrogen. After all the alkyl halide was added the temperature was raised to 500°C. with the nitrogen still flowing to sweep out the reactor.

Table I contains a summary of the best conditions found for each reactant, the products formed, and analyses of the products. Chlorosilyl compounds were analyzed by the method of Hyde and DeLong (9). Hydrogen attached to silicon was determined by Stock's method (20), and the resulting solution was then analyzed for silicon.

Reaction of Chloroform with Copper-Silicon

When 360 gm. of chloroform was passed over heated copper-silicon (340–360°C.), 100 gm. was recovered. Aside from a large quantity of silicon tetrachloride, the only product identified was 1.2 gm. boiling at 140–150°C. Perchlorodisilane boils at 145°C. (7).

Reaction of 2-Chloropropane with Copper-Silicon

When 210 gm. of 2-chloropropane was passed through the heated copper-silicon (360–410°C.) a large amount of liquid condensed in the Dry-Ice-cooled trap. At room temperature this evolved a gas which decolorized bromine in carbon tetrachloride. 1,2-Dibromopropane (63 gm.) was obtained by fractionation. This boiled at 138–142°C.; d , 1.938; n_D^{20} , 1.5195. The literature (7) gives the following constants: b.p., 140°C.; d , 1.933; n_D^{20} , 1.5203.

The reactions between chlorosilanes and lithium aluminum hydride were carried out in round-bottomed five-necked flasks. A dropping funnel, an indented West condenser for refluxing, a nitrogen inlet, and a thermometer were connected to the flask. There was a connection between the flask and the top of the dropping funnel to equalize pressure. To the upper end of the reflux condenser was attached a trap cooled in Dry Ice. The trap opened to the atmosphere through a calcium chloride drying tube. In Table II are listed the disilyl alkanes synthesized and the analytical results.

Synthesis of Disilylmethane

Reduction of 32 gm. of dichlorosilyltrichlorosilylmethane in 50 ml. ether by the addition of 24 gm. of lithium aluminum hydride in 250 ml. ether, followed by fractional distillation of the solution, yielded 5 gm. (52%) disilylmethane,

TABLE II
DISILYLALKANES

Compound	Yield, %	Analysis			
		% Silicon		% Hydrogen attached to silicon	
		Calc.	Found	Calc.	Found
Disilylmethane	0-80	73.4	73.6	7.9	4.0*
1,1-Disilylethane	8	—	—	6.7	6.6
1,2-Disilylethane	5	62.2	62.3, 62.6	—	—
1,2-Disilylpropane	Product not completely pure*				

*See text.

boiling at 26-27°C. This was a colorless, mobile liquid whose vapor ignited spontaneously in air. Once, while some of the vapor was being transferred, it was accidentally mixed with a small quantity of air and then slightly warmed (> 35°C.). The apparatus was completely shattered in the resulting explosion.

A sample weighing 0.0219 gm. was analyzed. The amount of hydrogen that should have been released was 38.6 ml. The amount actually released was 19.5 ml. or 0.504 of the theoretical amount. When the solution was acidified a further quantity of gas was evolved. This observation was not repeated as the rest of the disilylmethane was lost in the explosion described.

No product was obtained when excess lithium aluminum hydride solution was added to *bis*-trichlorosilylmethane. When the chlorosilane was added to the hydride an 80% yield was obtained.

Synthesis of 1,2-Disilylpropane

1,2-*bis*-Trichlorosilylpropane, 0.8 gm., was hydrogenated. Fractionation did not yield pure 1,2-disilylpropane. However, the fraction boiling where predicted by the Egloff equation, 94°C., contained more silicon than the fractions on either side (Table III). It is believed that the compound synthesized was 1,2-disilylpropane.

TABLE III
FRACTIONATION OF 1,2-DISILYLPROPANE

Boiling range (corrected), °C.	Weight, gm.	% Si	Weight Si, gm.
92-94	0.2	3.9	0.008
94-96	0.3	8.6	0.026
96-98	0.7	1.7	0.012

DISCUSSION OF RESULTS

The Rochow synthesis was found to be simple but yields were low. Temperature control was usually not critical in the range 300-400°C. as long as at least 300°C. was maintained. Preheating the copper-silicon mixture with hydrogen at 1000°C. did not affect the yields, nor did the rate of addition of the dichloroalkane. These findings are similar to those reported by Riccoboni and Zotta (14).

The relative positions of the chlorine atoms influenced the yields. 1,1-Dichloro compounds gave 8% yields, 1,2- compounds gave *ca.* 1% yields, and 1,4-dichlorobutane gave only a trace (0.01%) of 1,4-*bis*-trichlorosilylbutane.

In all experiments more silicon tetrachloride and silicon chloroform were produced than desired products and there were low boiling substances which did not contain silicon or labile chlorine. In the experiments with 1,2-dichloropropane the by-product decolorized a solution of bromine in acetic acid but no brominated substance could be isolated. When 2-chloropropane was the starting material an unsaturated substance was identified as propene from the dibromide. The low boiling by-products from experiments with dichloromethane and 1,1-dichloroethane did not decolorize bromine solutions. From these properties, it is likely that the by-products were hydrocarbons. These have previously been reported from the Rochow reaction (19).

The presence of silicon-hydrogen bonds in the products, e.g. dichlorosilyltrichlorosilylmethane from dichloromethane, the large quantities of chlorosilanes and hydrocarbons produced, and the low yields of the desired products all point to extensive decomposition of the dichloroalkanes. This could not be avoided, as, at temperatures causing less decomposition, less of the desired products were obtained.

An interesting point is that no products were identified in which only one of the original chlorine atoms had reacted to yield a chloroalkyltrichlorosilane.

The reaction of trichloromethane with copper-silicon to produce perchlorodisilane was similar to the synthesis of that compound from carbon tetrachloride (2, 12).

The physical properties of the compounds synthesized are summarized in Table IV.

All compounds were colorless when pure but the chlorine-containing ones decomposed to colored substances on standing. *bis*-Trichlorosilylmethane exhibited a blue fluorescence in ultraviolet light. A similar fluorescence was noted by Challenger and Kipping (4) with some of their alkylchlorosilanes. The completely hydrogenated substances had a musty odor, the lower chlorine-containing ones had the odor of hydrogen chloride, and the higher ones had a musty yet sharp odor.

Vapor pressures at several temperatures were measured for most of the compounds. Graphs were plotted of vapor pressure vs. temperature and log vapor pressure vs. reciprocal of absolute temperature. The former were smooth curves, the latter straight lines. The equations of the straight lines were calculated and are given in Table IV. The latent heats of evaporation and Trouton's constants were calculated from the slopes of the $\log p$ vs. T^{-1} plots. The Trouton values indicated that the chlorine-containing compounds were associated, while disilylmethane was not. This is similar to previous findings (1,6).

The only reference to dichlorosilyltrichlorosilylmethane in the literature (11) gives just one datum, b.p.₁₀ = 51.0–52.5°C. In this work the boiling point measured at atmospheric pressure was 167–168°C. Using the Ramsay-Young

TABLE IV
PHYSICAL PROPERTIES OF ORGANIC COMPOUNDS CONTAINING TWO SILICON ATOMS

Compound	B.p., °C. at 760 mm.	Density at $t^\circ\text{C.}$, gm./ml.	t_i , °C.	Vapor pressure equation ¹					Lv(calc.), cal./mol.	Trouton's constant, cal./mol./°K.
				Constants		Range, °K.	Error, ± °K.			
				A	B					
<i>bis</i> -Trichlorosilylmethane ^a ($\text{Cl}_3\text{SiCH}_2\text{SiCl}_3$)	180 ^b	1.545	17	-2380	8.1397	328-453	1.5	10,900	24.0	
Dichlorosilyltrichlorosilylmethane ($\text{Cl}_2\text{HSiCH}_2\text{SiCl}_3$)	167-168 ^d	1.521 1.464	23 27.5	-	-	-	-	-	-	
1,1- <i>bis</i> -Trichlorosilylthane ($\text{Cl}_3\text{SiCH}(\text{CH}_3)\text{SiCl}_3$)	191	1.454	27.5	-2425	8.1089	363-468	1.2	11,900	23.7	
1,2- <i>bis</i> -Trichlorosilylthane ($\text{Cl}_3\text{SiCH}_2\text{CH}_2\text{SiCl}_3$)	198 ^b 85(25mm.)	1.467	28	-2440	8.059	401-474	1.2	11,180	23.6	
1,2- <i>bis</i> -Trichlorosilylpropane ($\text{Cl}_3\text{SiCH}_2\text{CH}(\text{CH}_3)\text{SiCl}_3$)	175-180 (80 mm.)	-	-	-	-	-	-	-	-	
1,4- <i>bis</i> -Trichlorosilylbutane ($\text{Cl}_3\text{Si}(\text{CH}_2)_2\text{SiCl}_3$)	97-99 (5 mm.)	-	-	-	-	-	-	-	-	
Disilylmethane ($\text{H}_3\text{SiCH}_2\text{SiH}_3$)	28	0.754	20	-1370	7.4190	247-299	0.7	6240	20.8	
1,1-Disilylthane ($\text{H}_3\text{SiCH}(\text{CH}_3)\text{SiH}_3$)	56-57									
1,2-Disilylthane ($\text{H}_3\text{SiCH}_2\text{CH}_2\text{SiH}_3$)	67.0-68.5									
1,2-Disilylpropane ($\text{H}_3\text{SiCH}_2\text{CH}(\text{CH}_3)\text{SiH}_3$)	94-96									
		M.p. - 50°C. M.p. - 15°C. to - 14°C.								

¹ $\log p_{\text{mm.}} = AT^{-1} + B$.

² $n_D^{20} = 1.471 \pm 0.001$.

³ $B.p._{760} = 184-185^\circ\text{C.}$ (11), $b.p. = 185.1-185.5^\circ\text{C.}$ (18).

⁴ $B.p._{760} = 51.0-52.5^\circ\text{C.}$ (11).

⁵ $B.p. = 202^\circ\text{C.}$ (2), $b.p._{760} = 92.5-93^\circ\text{C.}$ (11).

M.p. = 50°C.
M.p. = 15°C. to -14°C.

formula (13) with *bis*-trichlorosilylmethane as the reference substance, the boiling point of dichlorosilyltrichlorosilylmethane was calculated to be 51°C. at 10 mm. pressure.

All compounds hydrolyzed very rapidly in alkali, forming curdy, brittle gels; those containing chlorine hydrolyzed slightly more rapidly than those without. The chlorinated compounds fumed in air. Glass apparatus dried at 110°C. for several hours still retained sufficient moisture to cause formation of polymer films.

Why disilylmethane evolved only 50% of the theoretical quantity of hydrogen when decomposed with alkali cannot be explained. The further evolution of gas when the solution was neutralized may indicate that a complex formed in the concentrated solution. Stock (20) used 30% sodium hydroxide while 60% potassium hydroxide was used in our work.

ACKNOWLEDGMENTS

One of the authors (W. D. E.) wishes to express his gratitude to the Department of Industry & Commerce, Province of Quebec for a special grant. Grateful acknowledgment is expressed to St. Lawrence Alloys & Metals Ltd., for donating and analyzing the silicon used in this research, to Canadian Copper Refineries Ltd., for donation of the copper, and to E. I. DuPont de Nemours & Co. Inc., for donating the 1,4-dichlorobutane.

Services rendered without charge by R. N. Taylor & Co., and Defence Industries Ltd., are appreciated. For the use of the facilities of the Defence Research Chemical Laboratories and the technical assistance of Mrs. M. M. English we are duly grateful.

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PAPER CHROMATOGRAPHIC SEPARATION OF SOME BIOLOGICALLY IMPORTANT PHOSPHATE ESTERS¹

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ABSTRACT

Any pair of the group of 11 phosphate esters studied may be separated on a paper chromatogram with one of three solvent mixtures: ethyl acetate - acetic acid - water, methyl Cellosolve - methyl ethyl ketone - ammonia water, ethyl acetate - formamide - pyridine. Separation of the components of more complex mixtures usually requires the use of two-dimensional paper chromatography. Position constants for the individual esters with each of the three solvents are listed, and diagrams showing the type of separation pattern obtained by two-dimensional chromatography of mixtures are presented.

INTRODUCTION

Current views on the mechanism of photosynthesis assume the intermediation of various phosphate esters such as those of the Embden-Meyerhof scheme (1, 3). A relatively simple, semimicro technique for the detection and identification of these compounds would therefore be desirable. Paper chromatographic separation of the phosphate esters has been the subject of an extensive and methodical study made by Hanes and Isherwood (5). Their results pointed to the need for a two-dimensional system, since it appeared unlikely that a single solvent could effect adequate separation of all the esters of biological importance. Procedures used by Aronoff (1), Benson *et al.* (3), and Cohen *et al.* (4) for particular groups of phosphate esters were not generally applicable to the separation of the hexose phosphates. Bandursky and Axelrod (2) report the separation of phosphate esters by two-dimensional ascending chromatography after dividing the esters into two groups by barium precipitation. The objective of these authors was essentially the same as that of the present study but different techniques and solvents were employed.

The goal of the experiments here reported was the development of three chromatographic solvent mixtures which would produce unique position patterns for the group of phosphate esters and to apply these in combinations to two-dimensional chromatograms.

EXPERIMENTAL

Apparatus

One-dimensional runs were carried out in Pyrex glass cylinders (8½ in. I.D. × 18 in.). Rectangular battery jars (12 × 13 × 19½ in. high) were used for two-dimensional runs. Both types were fitted with glass racks for descending chromatography. Troughs were made from sections of 40 mm. Pyrex tubing, sawed lengthwise. The solvent lift distance (surface of the solvent to the top of the parallel glass rod) was 1 to 2 cm. All solvents except ammonia water were glass distilled; fractions coming over within two degrees of the respective boiling points were saved.

¹ Manuscript received April 4, 1952.

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Preparation of the Paper

Strips and squares of Whatman No. 1 filter paper cut from $18\frac{1}{4} \times 22\frac{1}{2}$ in. sheets were used throughout. Purification methods were based on those used by Hanes and Isherwood (5). To facilitate the prewash operation, a perforated filter plate (11 \times 18 in.) was constructed from sheet Lucite. The boxed-in space below the plate was provided with a hole for connection to a vacuum line. The wash consisted of two 1-liter portions of *N* hydrochloric acid followed by six 1-liter portions of distilled water for batches of 20 sheets. The alcoholic 8-hydroxyquinoline wash used by Hanes and Isherwood (5) was omitted or replaced by an aqueous wash of 0.02% Versene.*

Preparation of the Sample

Except for glycerol-1-phosphate† and 2,3-diphosphoglyceric acid,‡ compounds were obtained as barium salts from commercial sources and used without additional purification. Solutions of approximately 0.1 *M* concentration were prepared by removing barium with saturated potassium sulphate or ammonium sulphate solution in 0.5 *N* hydrochloric acid, and adjusting the pH with the corresponding base. At pH 5 (bromcresol green end point) with storage at 4°C., the solutions were stable for six weeks. At higher pH values, deterioration was considerably accelerated. Under such conditions, fructose-6-phosphate underwent a Lobry de Bruyn type transformation to glucose-6-phosphate. Solutions deionized with cationic exchange resins gave more diffuse spots than those resulting from solutions of potassium or ammonium salts. Chemically pure reagents were used to avoid introducing traces of heavy metals into sample solutions.

Preparation of the Chromatogram

The jar was prepared at least a day in advance by placing in the bottom a one-phase mixture of the solvent components. To hasten vapor equilibration, sheets of filter paper dipping into the mixture extended up two sides of the jar to the top. The sample was spotted on the paper with a capillary pipette. Two to six μ liters of a 0.1 *M* solution of the sample contained enough phosphorus to be detected on the developed chromatogram. The higher level was necessary for the detection of glucose-6-phosphate and 3-phosphoglyceric acid. Quantities in excess of these amounts produced large spots on most chromatograms. The filter paper sheet or strip was arranged in the chamber about two hours before addition of the solvent to the trough. Paper clips at the bottom of the sheet improved drainage of the solvent from the paper. The glass lid to the jar was sealed on with stopcock grease. At the end of the run (16 to 24 hr.), the paper was hung in a hood to dry and finally in a drying oven at 85°C.

When the paper was to be used for a two-dimensional chromatogram, oven drying was omitted. The paper was kept in a well-ventilated hood for seven to eight hours before it was placed in the second jar. Chromatograms started in the late afternoon ran in the first direction overnight. The paper was dried during the next day and was ready for overnight development in the second direction by late afternoon.

*Bersworth Chemical Co., Framingham, Mass.

†Glycerol-1-phosphate was provided by Dr. M. Kates, 2,3-diphosphoglyceric acid by Dr. E. Baer.

Temperature variations apparently had very little effect on any of the systems studied, other than to influence the rate of flow of the solvent. The jar for the acidic solvent was kept in a room at 4°C. to reduce the rate of flow, and the other jars were kept at room temperatures (23–27°C.). Conditions governing pre-equilibration of the paper were similarly not critical. With the solvents here reported, in the apparatus specified, no significant difference could be detected between chromatograms pre-equilibrated 2 or 24 hr. For periods of less than two hours occasional erratic chromatograms did appear, probably because opening the jar had disturbed the vapor phase.

Successful use of the ethyl acetate–pyridine–formamide solvent required relatively dry conditions. For the experiments reported, the papers, jar, and solvent were in equilibrium with an atmosphere having a relative humidity of 20 to 35% at 25°C. When the atmosphere in the jar contained higher concentrations of water vapor, subsequent chromatograms showed poor resolution and an altered order of travel for some of the compounds.

Detection of the Compounds

Various sprays for the detection of phosphate were examined. The most useful reagent (Hanes and Isherwood (5)) contained molybdate, perchloric acid, and hydrochloric acid. The sprayed paper was placed in an oven at 85°C. for five to six minutes, then transferred to a jar containing water vapor and a little hydrogen sulphide. This procedure gave faint spots for the stable esters, glucose-6-phosphate, and 3-phosphoglyceric acid. If, however, the paper was first sprayed with aniline phthalate in butanol (6) and heated, glucose-6-phosphate and fructose diphosphate appeared as brown spots. Subsequent spraying with the molybdate solution brought out the blue spots for the other esters, including phosphoglyceric acid.

The method of detection introduced by Bandursky and Axelrod (2) was used successfully for a few of the final experiments. The paper was sprayed with the Hanes and Isherwood reagent, dried at room temperature, and placed under a 15-watt ultraviolet germicidal lamp. Blue spots appeared almost immediately. A number of modifications of the reagent were tested but the original mixture was apparently optimal. Most changes in composition caused an increase in background color under ultraviolet light. With this technique, it was essential to remove all solvent from the paper. Low temperature (85°C.) oven drying was necessary with some of the solvents used.

Measurements

Positions of zones were measured from the leading edge of the spot. This edge was usually well defined, and measurements at different concentrations did not change so much as those based on the mid-point of the spot. Since the solvent front normally ran off the end of the paper, the position occupied by orthophosphate was used as the point of reference. The positions of the other spots were given values proportional to orthophosphate as 100. The values thus derived, that is, $\frac{\text{distance traveled by the compound} \times 100}{\text{distance traveled by orthophosphate}}$, will be referred to as position constants.

RESULTS

The investigations of Hanes and Isherwood (5) indicated that improvement in the separation of the hexose phosphates was desirable. Since these authors had found similar patterns for most acidic solvent mixtures, an intensive search for a new solvent was not considered necessary. Relatively few solvents were tested, but improvement in the resolution was sought by varying the composition of the mixtures and the conditions of use. The actual separation of two spots on the chromatogram depended both on position and size of the spot. The work reported was directed toward increasing the difference between measured positions without contributing to the spreading of the spots.

A group of the lower primary and tertiary alcohols, ketones, esters, and ethers was studied in combination with formic, acetic, propionic, and butyric acids, benzenesulphonic acid, ammonia, pyridine, morpholine, formamide, β -chloroethanol, propylene glycol, and methyl Cellosolve. Water was, of course, an essential component of most mixtures.

Poor resolution on the chromatogram has been related previously (5) to increased water content of the solvent. As data accumulated, this observation was confirmed and, in addition, it was found that diffuse and poorly defined spots were associated with the presence of alcohols in the solvent mixtures. In an attempt to develop a solvent containing neither water nor alcohols, formamide was substituted for water. The resultant solvent produced chromatograms with compact, well defined spots. The relative positions of the esters on such chromatograms were also different from those obtained with the acid and alkaline solvents.

The three solvents finally selected as most useful for the separation of phosphate esters were: (a) ethyl acetate 3, acetic acid 3, water 1; (b) methyl Cellosolve 7, methyl ethyl ketone 2, 3 *N* ammonium hydroxide 3; (c) ethyl acetate 6, formamide 4, pyridine 1, (or 1:2:1). R_f values and position constants of the group of phosphate esters for each of these solvents are given in Table I. The solvent front traveled about 30 cm. for determination of the R_f values, and the fastest moving component of the group about 25 cm. for calculation of the position constants. Ideally, the position constant does not depend upon the position of the solvent front. Actually, a degree of dependence was observed for the two solvents containing water. With increasing length of run the position constants for the hexose phosphates decreased in the acid solvent and usually increased in the basic solvent. The magnitude of the effect was not great enough to cause difficulty in identifying the compounds.

The R_f values under conditions of optimum resolution were usually low (Table I). By running the solvent off the paper, the theoretical length of the chromatogram was extended to about two and one-half times the actual length of paper. The esters were then sufficiently separated to permit identification. Any pair of the esters listed in Table I was separable by one of the solvents. With increasing complexity of mixture, greater difficulty was encountered in obtaining separation of glucose-1-phosphate from glucose-6-phosphate. Other phosphates in mixed samples chromatographed with the acid solvent caused glucose-1-phosphate to

TABLE I
 R_f VALUES AND POSITION CONSTANTS FOR SOME PHOSPHATE ESTERS IN DIFFERENT SOLVENT MIXTURES

	Ethyl acetate Acetic acid Water 4°C.		Methyl Cellosolve Methyl ethyl ketone 3 <i>N</i> ammonium hydroxide 26°C.		Ethyl acetate Formamide Pyridine 26°C.	
	R_f^*	P. constant*	R_f	P. constant	R_f	P. constant
Orthophosphate	0.33	100	0.21	100	0.50	100
Glycerol-1-phosphate	0.26	79	0.39	192	0.54	114
Glyceraldehyde-3-phosphate	0.07	22	0.19	90	—	—
2-Phosphoglyceric acid	0.27	81	0.41	200	0.23	47
3-Phosphoglyceric acid	0.23	71	0.22	116	0.28	57
2,3-Diphosphoglyceric acid	0.11	35	0.07	36	0.15	30
Ribose-1-phosphate	0.15	45	0.40	197	0.50	110
Glucose-1-phosphate	0.14	37	0.36	170	0.44	89
Glucose-6-phosphate	0.12	29	0.29	140	0.50	100
Fructose-6-phosphate	0.17	48	0.36	171	0.54	108
Fructose-1,6-diphosphate	0.08	25	0.08	37	0.13	26

* $R_f = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent front}}$

P. constant = $\frac{\text{Distance traveled by compound} \times 100}{\text{Distance traveled by orthophosphate}}$

R_f values and position constants were calculated from different data.

form an elongated spot overlapping the position occupied by glucose-6-phosphate. The ratio of position constants was used as an index of the degree of separation of various pairs of compounds. If the ratio for a given pair was greater than 1.25, separation on the chromatogram was indicated. For example, the ratio of F6P:G6P for each of the solvents in Table I is: 1.65, 1.22, and 1.08. The two esters were thus well separated by the acid solvent (1.65), partially separated by the basic one (1.22), and not separated by the formamide solvent.

Since each of the solvents was a one-phase mixture, considerable variation in the relative proportion of components was possible. The effects of one such series of variations are given in Table II. As the proportion of ethyl acetate in the mixture was increased, the position constant ratio of F6P:G6P increased and passed through a maximum of 1.65. The ratio for G1P:G6P, on the other hand, remained near 1.0. These data are useful to illustrate that changes in composition within a solvent mixture do not necessarily bring about parallel alterations in the R_f values for all the compounds being chromatographed. In fact, inversion of order occurred with G6P and HDP. The solvent selected does not necessarily provide optimum separations for all components of the mixture. In the present study the solvent which gave the best separation of fructose-6-phosphate and glucose-6-phosphate was used.

TABLE II
POSITION CONSTANT RATIOS SHOWING THE EFFECT ON RESOLUTION
OF VARIATIONS IN SOLVENT COMPOSITION

	Solvent composition, parts by volume, ethyl acetate:acetic acid:water				
	2:6:1	2:3:1	3:3:1	5:3:1	7:3:1
F6P* G6P	1.23	1.36	1.50	1.65	1.46
G6P HDP	0.81	1.13	1.00	1.22	1.37
G1P G6P	1.06	1.02	1.05	1.03	1.27

*Abbreviations are listed under Fig. 1.

Tracings of completed two-dimensional chromatograms of mixed samples are shown in Fig. 1. The chromatogram for Fig. 1 (left) was the type produced with ethyl acetate - acetic acid - water and ethyl acetate - formamide - pyridine as solvents (system 1). The pattern on the second sheet (Fig. 1, right) was produced when methyl Cellosolve - methyl ethyl ketone - ammonia water and ethyl acetate - formamide - pyridine were employed (system 2). The spots on chromatograms from system 1 were remarkably well defined. Although glucose-1-phosphate and glucose-6-phosphate were only partially separated, identification of each was possible. If the paper was first sprayed with aniline phthalate and heated, the 6-phosphate showed as a brown spot. Respraying with the molybdate reagent in the usual manner brought out a blue area with a different shape from the original spot. The change in shape was caused by glucose-1-phosphate.

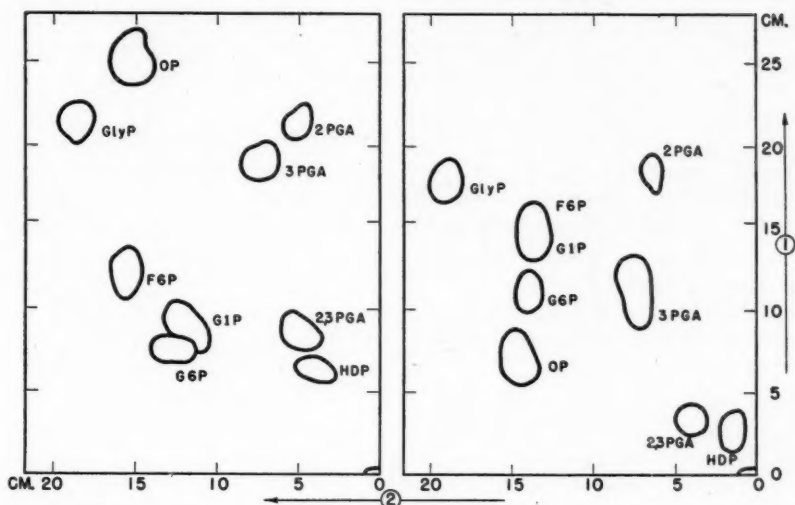


FIG. 1. Two-dimensional chromatogram tracings.

Left—Solvents: Ethyl acetate - acetic acid - water (3:3:1), followed by ethyl acetate - formamide - pyridine (6:4:1).

Right—Solvents: Methyl Cellosolve - methyl ethyl ketone - 3*N* ammonium hydroxide (7:2:3), followed by ethyl acetate - formamide - pyridine (6:4:1).

Direction and order of solvent flow are indicated by the numbered arrows.

In both cases, 5 μ liters of sample was applied at the origin. The sample contained about 20 mgm. per ml. of each of the following: inorganic orthophosphate (OP), glycerol-1-phosphate (GlyP), 2-phosphoglyceric acid (2PGA), 3-phosphoglyceric acid (3PGA), 2,3-diphosphoglyceric acid (2,3PGA), glucose-1-phosphate (G1P), glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose-1,6-diphosphate (HDP).

The spots on chromatograms of system 2 were much more diffuse than those of system 1. However the positions of the compounds on the sheet were sufficiently different to make the system useful for confirming the identification of individual esters.

Other combinations of the three solvents did not produce satisfactory two-dimensional chromatograms, because the spots on such sheets were too diffuse and poorly defined.

DISCUSSION

With different acidic solvent mixtures, one-dimensional chromatograms gave similar patterns. Inorganic phosphate and the three carbon esters traveled well ahead of the hexose monophosphates, and the diphosphorylated compounds traveled most slowly. The particular order of the compounds within the groups varied with the composition of the solvent. For example, an alcohol in combination with an acid and water caused fructose-6-phosphate and glucose-1-phosphate to travel at the same rate, ahead of glucose-6-phosphate. When the alcohol was replaced by an ester, fructose-6-phosphate preceded the unresolved glucose phosphates. In some solvents, such as those containing ketones, fructose diphosphate lagged well behind glucose-6-phosphate, while in the ester-acid-

water combinations, both had similar rates. With certain combinations of ester-acid and water (Table II), the diphosphate traveled faster than the 6-phosphate. These shifts of position were not large but permitted choice in the type of separation to be effected.

Only two combinations of the three solvents gave useful two-dimensional chromatograms. With the other systems the spots on developed chromatograms were diffuse or streaked. Apparently the passage of the first solvent through the paper influenced the results obtained with the second solvent. An example of this effect is shown in Fig. 1. Although the same solvent was used in the second direction for both systems, the resultant patterns in this direction did not coincide. Some structural change in the paper brought about by the first solvent may have been responsible for this difference and also for the diffuse spots.

The position of an ester on the chromatogram was not changed when the compound was chromatographed in a mixture of phosphate esters, but the size of the spot was usually increased. The degree of separation between two close-running compounds was thus usually less with mixed samples than would be predicted from the spot size and position of each compound run separately. This condition may be caused by overloading the paper with phosphate. While the resultant spreading does interfere with the separation of mixtures on one-dimensional chromatograms, it is not important with two-dimensional systems because the compounds (and thus the phosphate) are scattered.

The size and clarity of the spots on chromatograms were determined to a considerable extent by the composition of the solvent mixture. The use of aqueous, acid or alkaline, alcohol mixtures, such as those employed by Bandursky and Axelrod (2), resulted in diffuse spots with indistinct borders. When esters or ketones were substituted for the alcohols, some improvement was obtained. The clearest spots were produced by a further substitution of formamide for water. It was therefore possible to select solvent components which would combine minimum spreading of the spots with sufficient separation for identification of the phosphate esters.

ACKNOWLEDGMENT

The author is grateful for the suggestions and encouragement given by Dr. K. A. Clendenning.

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THE POLAROGRAPHIC DETERMINATION OF TITANIUM IN STEELS AND NICKEL-BASE ALLOYS¹

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ABSTRACT

Titanium in steels and nickel-base alloys can conveniently be determined polarographically after electrolysis of a solution of the alloy in a mercury-cathode cell. A well-formed wave, the height of which is directly proportional to the concentration of titanium in the solution, is recorded using a supporting electrolyte that is 1.0 *M* in tartaric acid, 0.5 *M* in sulphuric acid, and 1.2 *M* in ammonium sulphate.

The method is one of good precision and yields values in excellent agreement with those given by umpire methods for samples of basic open-hearth steel, 18-11 chromium-nickel steel, Monel, Inconel "W", and Nimonic 75 and 80.

INTRODUCTION

The only earlier work on the polarographic determination of titanium in metallurgical products appears to be that of Zan'ko, Geller, and Nikitin (25) who proposed a method for the determination of this element in cast irons and steels. They report the determination of titanium in an acid tartrate medium after treating a solution of the alloy with aluminum dust. We have not been able to remove the interference of iron (III) by the use of this reducing agent; furthermore, the introduction of an appreciable concentration of aluminum ion is undesirable (3).

Recent work in this laboratory on the polarographic determination of titanium in rocks and minerals (4) has shown that very good polarographic waves for titanium are obtained in a medium containing tartaric acid, sulphuric acid, and ammonium sulphate. Interfering ions are removed by prior electrolysis of the solution in a mercury-cathode cell. The application of such a method to the determination of titanium in steels and nickel-base alloys is discussed in the present paper.

REMOVAL OF INTERFERENCES

Because the half-wave potential for copper (II) in an acid tartrate medium is less negative than that of titanium, there is a limit to the concentration of copper that can be present without the occurrence of interference in the polarographic determination of titanium. Iron (III) also constitutes an interference (23). Electrolysis in a mercury-cathode cell is a very convenient and efficient way of removing from solution iron, copper, and nickel—and a score of other elements (12)—all without the introduction of foreign salts, and often in less time than that required by other procedures. In such an electrolysis chromium is not easily removed quantitatively but the chromium remaining in solution causes no interference in the determination of titanium, even in alloys high (20%) in chromium. The chromium content of the solution being polarographed should be

¹ Manuscript received February 25, 1952.

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limited, however, for otherwise there may be a significant depression of the diffusion current for titanium (16). We found an 8% effect when the molar ratio $[\text{Cr}^{+++}]/[\text{Ti}^{++++}]$ was 100, although there was no significant depression when the ratio was 10. Neither chromium nor nickel yields a wave in the supporting electrolyte that we used. The half-wave potential of chromium (III) in (saturated) tartaric acid solution has been reported as about -1.0 volt vs. the saturated calomel electrode (7, p. 291); possibly the wave was merged with that for the discharge of our supporting electrolyte which occurred at about this potential.

In 1903, in one of the relatively early papers concerned with the use of the mercury-cathode cell, Smith stated (21) that "quantitative separations of iron from titanium, from zirconium and from thorium were made and the results were most satisfactory". Electrolysis in a mercury-cathode cell has subsequently been used widely to separate interfering elements before the determination of titanium by colorimetric or gravimetric methods, e.g., in the determination of titanium in ores (18), in rocks and minerals (4), in ferro-titanium (19), in nickel-base alloys (13), in chromium steels (6), and in high-temperature (20) and other alloys (2, 22). We have made careful wave-height measurements on polarograms given by both electrolyzed and nonelectrolyzed aliquot portions of a solution of titanium and the data (240 wave-height measurements by two readers) confirm the view that titanium is quantitatively retained in the electrolyte during electrolysis with a mercury cathode.

The rate at which iron is deposited in a mercury-cathode cell is favorably affected by the use of fresh mercury, a low concentration of sulphuric acid, agitation of the mercury, and by the proper choice of spacing for the anode relative to the cathode (1, 5, 14, 15, 17). The rate of iron removal increases with increasing current density, but care must be taken to prevent undue boiling of the solution. We studied the desirability of removing much of the iron from samples of steel by an ether extraction (24, p. 51) with a view to shortening the time of electrolysis. It was found, however, that the inconvenient and time-consuming nature of the ether extraction was such as to make its use inadvisable.

APPARATUS AND REAGENTS

An Heyrovský polarograph, Model XI (E. H. Sargent and Co.) was used throughout this work. The capillary was cut from special tubing supplied by E. H. Sargent and Co., and was cleaned, when necessary, by immersing it in hot nitric acid while the mercury was flowing (7, p. 243).

The m and t values of the capillary in the supporting electrolyte (1.00 M tartaric acid, 0.50 M sulphuric acid, 1.20 M ammonium sulphate) were determined at $25.0 \pm 0.1^\circ\text{C}$. by the method of Lingane and Kolthoff (9). At an applied potential of -0.30 volt vs. the saturated calomel electrode, the drop time, t , was 5.07 sec., the rate of flow of mercury, m , was 1.357 mgm. per sec., and $m^{2/3}t^{1/6}$ was 1.606 $\text{mgm.}^{2/3}\text{sec.}^{-1/2}$. (The variation of $m^{2/3}t^{1/6}$ with applied potential was in close agreement with the relative values given by Kolthoff and Orlemann (8).)

The polarographic cell used was of the H-type, similar to that described by Lingane and Laitinen (10), one arm of which contained the saturated calomel reference electrode.

The mercury-cathode cell was constructed from a wide-necked, 250 ml. Erlenmeyer flask. The bottom of the flask was blown to resemble a Kjeldahl flask and a three-way stopcock was attached as shown in Fig. 1. The leveling bulb was used to adjust the volume of mercury in the cell and to facilitate removal of spent mercury. The outlet *A* was for the removal of the electrolyte from the cell on the completion of electrolysis. Through a two-holed rubber stopper were inserted a platinum anode, formed into a flat spiral which was parallel to the mercury surface, and a short-stemmed funnel which was covered with a watch glass in order to prevent spray losses during electrolysis.

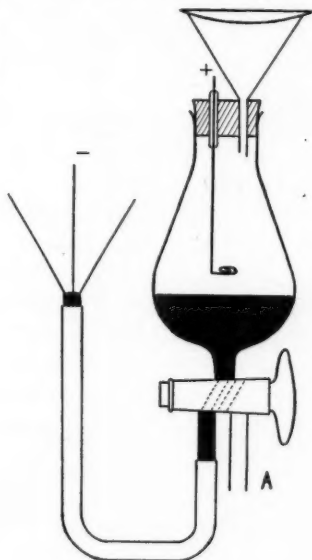


FIG. 1

The mercury used for the mercury cathode was purified by bubbling for 10 hr. with 10-15% nitric acid followed by several washings with water. Mercury for the dropping electrode of the polarograph was further purified by two distillations *in vacuo*.

All reagents were tested for the presence of titanium (IV) ions, both colorimetrically and polarographically, with negative results.

METHOD OF ANALYSIS

The weight of sample taken depends on the concentration of titanium and the polarographic galvanometer sensitivity chosen. Working at $R = 10$ with our instrument, concentrations of titanium, in the final solution, of from 0.005 to

0.07 mgm. per ml. (or $1.0 \times 10^{-4} M$ to $1.4 \times 10^{-3} M$) could be measured conveniently, but we preferred the concentration to be about 0.05 mgm. per ml. (On the basis of a 1 gm. sample in a final volume of 100 ml., this corresponds to 0.5% of titanium.)

Dissolution

A sample of 1 to $2\frac{1}{2}$ gm. was weighed accurately, treated with a solution of acids,* and heated gently to effect solution; about 15 ml. of acid solution was found sufficient for a 1 gm. sample. After the sample had completely dissolved, 25.0 ml. of 5.0 *M* sulphuric acid was added and the solution evaporated to fumes, great care being taken to avoid spattering as the solution approached a syrupy condition. The sample was then treated with about 20 ml. of water and the solution and any residue transferred carefully to the mercury-cathode cell, and diluted to about 75 ml.

Electrolysis

The solution was electrolyzed at 4 to 6 volts, using about 90 ml. of pure mercury in the cell and a current density of 0.20 to 0.27 amp. per sq. cm. Electrolysis was carried out for not more than one hour, and was discontinued within this time if a spot test for iron (using 2,2'-dipyridyl or potassium thiocyanate) became negative. The electrolyte was quantitatively removed from the cell, filtered through Whatman No. 40 paper, and evaporated to about 50 ml.

Polarography

The solution was then made just basic to litmus using 15 *M* aqueous ammonia; 25.0 ml. of 2.0 *M* sulphuric acid and 15.0 gm. of anhydrous tartaric acid were added, and the solution was diluted to 100.0 ml. and mixed thoroughly.** A portion of the solution was decanted into the polarographic cell, bubbled for 15 min. with nitrogen (purified by passing the gas through a solution of chromous sulphate (11), or an alkaline solution of potassium pyrogallate, and then distilled water), and then polarographed at $25.0 \pm 0.1^\circ C$. from 0.0 to -0.8 volt vs. the saturated calomel electrode. The bridge potential was 2.0 volts and the galvanometer sensitivity, $R = 10$. Each solution was polarographed four times and each of the four polarograms, which were recorded photographically on Kodabromide E-2 paper, was measured twice by the slope-intercept method.

The concentration of titanium was determined by reference to a straight-line calibration curve relating the concentration of titanium to wave height. The calibration was carried out using solutions prepared from Bureau of Standards titanium dioxide No. 154 and containing ammonium sulphate (1.20 *M*), sulphuric acid (0.50 *M*), and tartaric acid (1.00 *M*).

*One part, by volume, of 12 *M* hydrochloric acid to one part of 16 *M* nitric acid for the steels, Inconel and Monel samples, and three parts of 12 *M* hydrochloric acid to two parts of 16 *M* nitric acid for the Nimonic samples.

**If this would have yielded a solution containing titanium at a concentration appreciably greater than 0.05 mgm. per ml., then, before being made basic, the solution was diluted to 100.0 ml., and an appropriate aliquot taken. This was made basic to litmus with 15 *M* ammonia, and then there were added 25.0 ml. of 2.0 *M* sulphuric acid, 15.0 gm. of tartaric acid, and solid ammonium sulphate equivalent to the sulphate lost by the taking of the aliquot sample. The solution was then diluted to 100.0 ml.

Concentration of Ammonium Sulphate

The procedures outlined above for the preparation of the solution to be polarographed would yield a solution 1.25 *M* in ammonium sulphate if there had been no loss of sulphate during the evaporation to fumes of sulphuric acid. There is of necessity some loss, and it is for this reason that the concentration of ammonium sulphate in the supporting electrolyte used in the calibration experiments was slightly less than 1.25 *M*, namely 1.20 *M*. From one alloy sample to another, the loss of sulphate on fuming is no doubt variable, and thus the final solutions vary in their concentrations of ammonium sulphate. The effect of such

TABLE I
EFFECT OF CONCENTRATION OF AMMONIUM SULPHATE ON WAVE HEIGHT
 $\text{H}_2\text{SO}_4 = 0.50 \text{ M}$; $\text{H}_2\text{C}_4\text{H}_4\text{O}_6 = 1.00 \text{ M}$; $\text{Ti} = 0.0535 \text{ mgm. per ml.}$

Concn. of $(\text{NH}_4)_2\text{SO}_4$, <i>M</i>	Wave height*, mm.
0.80	60.5 \pm 1.1
1.00	59.5 \pm 1.3
1.20	58.7 \pm 1.5

*Each value is the average, with maximum deviation, of eight measurements—two on each of the four polarograms recorded for each solution.

variation on the wave height of the titanium wave, other factors being constant, was studied, with the results shown in Table I. There appears to be a slight increase in the diffusion current with decreasing concentration of ammonium sulphate, but the effect is small; a 20% variation in the concentration either way from 1.00 *M* causes a change in the diffusion current of only about 1.5%.

ACCURACY AND PRECISION

The accuracy of the proposed method is attested by the data of Table II, in which the polarographic analyses for titanium in steels and nickel-base alloys are compared with colorimetric analyses carried out by umpire methods in the Huntington Works Laboratory of The International Nickel Company (13) and at, or for, the National Bureau of Standards. The value of 0.20% for National Bureau of Standards Sample No. 162 is the average of determinations in seven

TABLE II
COMPARISON OF POLAROGRAPHIC AND COLORIMETRIC METHODS (% Ti)

Sample	"Certified" value	Polarographic value
International Nickel Co., Inc.		
Nimonic 75	0.40	0.395 \pm 0.004
Nimonic 80	2.40	2.44 \pm 0.07
		(2.42 \pm 0.02)*
Inconel "W"	2.32	2.35 \pm 0.04
Bureau of Standards		
Monel No. 162	0.20	0.196 \pm 0.001
18-11 Chromium-nickel Steel No. 121b	0.416**	0.449 \pm 0.008
Basic open-hearth Steel No. 170	0.23**	0.218 \pm 0.001

*Omitting one high value from five determinations.

**Provisional Certificate value; method probably colorimetric.

different laboratories (spread, 0.19–0.21%). The "certified" values for the Inconel and Nimonic samples are each averages of 5–10 separate determinations, which agreed closely (13).

The polarographic values recorded in Table II are each averages of at least three, and sometimes five, separate determinations; the precision measure shown is the maximum deviation from the average. In each polarographic determination four polarograms were recorded, and each polarogram was measured twice. The average of the eight wave-height measurements was taken as the value for the determination. The maximum deviation of a wave-height measurement from the average of eight varied from sample to sample within the range 0.8% to 2.7% of the average value. The reproducibility of waves and of their reading is therefore good, and, as shown in Table II, so also is the precision of repeated determinations.

More information about the precision of the method was given by the taking of 240 readings by two readers on 12 polarograms. These involved 10 measurements, by each reader, of the height of each of the 12 waves, four from each of three different aliquot samples of the same solution. For the 24 sets of 10 measurements on waves about 53 mm. in height, the standard deviations ranged from 0.2 to 0.7 mm. (with an average standard deviation of less than 0.5 mm.), the average deviation from 0.1 to 0.6 mm., and the maximum deviation from 0.3 to 1.6 mm. When the results were considered in groups of 40, i.e., all the results for a given aliquot grouped together, the standard deviations in the six sets of this kind of measurement ranged from 0.7 to 1.1 mm., with an average standard deviation of 0.8 mm. These deviations are akin to those found for similar measurements in an earlier study (4).

APPLICATIONS

The method is suitable for alloys of widely different composition. In the samples studied, the iron content ranged from less than 1 to more than 99%, the nickel from less than 0.1 to more than 70%, the chromium from less than 0.1 to 20%, the copper from 0.02 to almost 30%, the manganese from 0.2 to over 2%, and the aluminum from 0.03 to over 1%. (See Table III in which are given the percentages of the constituents, other than titanium, which are present in one alloy or another to an extent greater than 0.1%.) We have successfully analyzed samples of stainless steel type 321 by this method and have found it applicable to a wide variety of rocks and minerals (4). We are now investigating the usefulness of the method in the analysis of high-temperature alloys.

TABLE III
COMPOSITION OF ALLOYS (%)

Sample	Si	Mn	Cu	Ni	Co	Cr	Al	Fe
Steel (B. of S. No. 170)	0.06	0.23	0.10	0.04	—	0.04	0.03	~ 99.2
Steel (B. of S. No. 121b)	0.60	1.52	0.12	11.14	—	17.68	—	~ 68.3
Inconel "W"	0.11	0.53	0.02	~ 75.5(Ni + Co)	—	15.12	0.56	5.82
Nimonic 75	0.56	0.50	0.04	~ 73.3(Ni + Co)	—	20.42	0.15	0.51
Nimonic 80	0.22	0.60	0.03	~ 75.0(Ni + Co)	—	20.14	1.07	0.47
Monel (B. of S. No. 162)	0.67	2.34	28.93	66.38	0.54	0.23	0.23	0.34

There has recently been developed a method for the determination of titanium in aluminum-base alloys using this same supporting electrolyte, but without any prior mercury-cathode separation (3), and Potts (16) has used a similar supporting electrolyte for the determination of titanium in paint pigments.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to the Research Council of Ontario and to the Defence Research Board for the financial support that made possible this investigation.

We are also much indebted to Dr. A. D. Middleton, of The International Nickel Company, Inc., Huntington, W. Va., for his courtesy in supplying carefully analyzed samples of nickel-base alloys, and to Messrs. J. A. C. Shaw and R. Nishikawa for assistance with some of the experiments.

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THE PREPARATION OF CHLORITE HOLOCELLULOSE¹

BY J. H. E. HERBST

ABSTRACT

Mildly acid sodium chlorite solutions were allowed to act on white birch and black spruce sawdust at room temperature for various times; the insoluble fraction and the soluble nondialyzable fraction were isolated and weighed. If chlorite acts selectively on lignin the sum of these two fractions in time approaches true holocellulose. Chlorine and methoxyl determinations and examination of the ultraviolet absorption spectra showed that this was only approximately true. A small amount of carbohydrates was probably lost, and evidence was found for the existence of a chlorite-resistant lignin fragment in the soluble nondialyzable fraction.

INTRODUCTION

Whether chlorine dioxide or chlorite are capable, under certain conditions, of removing lignin quantitatively from wood without causing the loss of any polysaccharide material has long been a matter of interest to wood chemists. Holmberg and Jahn (4, 5) found that pentosan was lost throughout the reaction, and especially during the removal of the last few percentages of lignin. On the other hand, Jayme *et al.* (6, 7, 8, 9) obtained the corrected theoretical yield of holocellulose (weight of wood - weight of lignin in wood + weight of lignin in holocellulose) if the reaction was stopped at the appropriate time, i.e., when the holocellulose contained about 3% residual lignin. However, further investigation of this reaction by Jayme, Eser, and Hanke (7, 9) revealed the presence of additional polysaccharides in the liquors and led to the formulation of the "excess polysaccharide" hypothesis. A further series of experiments by Jayme and Fink (8) with varying proportions of chlorite demonstrated that the excess yield (actual yield - corrected theoretical yield) passed through a maximum as the proportion of chlorite was increased. Hydrolysis of these holocelluloses and subsequent reducing sugar determinations showed that the excess yield did not consist of polysaccharide but was probably due to the fact that the oxidized lignin lacks the ability to condense during the determination of lignin. Wise *et al.* (23, 24) confirmed Jayme's quantitative recovery in the case of softwoods; they were able to obtain satisfactory summative analyses using fractions extracted from holocellulose by alkali. With hardwoods, however, some losses occurred; the authors state that the holocellulose contains a substantial quantity of alcohol extractable matter and therefore the lignin determinations carried out on it cannot be used to calculate the weight of carbohydrates in the holocellulose.

The excess polysaccharide problem was further explored in a series of papers by Müller who found (11, 12) that under certain conditions the holocellulose prepared with chlorine and chlorine dioxide and obtained in greater than theoretical yield gave more reducing matter on hydrolysis, based on the wood, than the wood itself. It was further shown (13) that the excess carbohydrate was not a pentosan. Müller thus concluded that the polysaccharidelike excess material originated from the lignin. The acetyl content of the holocelluloses, based on the

¹ Manuscript received April 28, 1952.

Contribution from the Department of Chemistry, Ontario Research Foundation, Toronto, Ont.

wood, was found to be considerably higher than that of the wood itself, while the methoxyl content was higher than that calculated for the carbohydrate and lignin constituents in the holocellulose (14).

Ritman stated in a review (17) that the phenomena observed are due to the formation of lignin degradation products which become soluble during the lignin determination; the quantity of lignin degradation products goes through a maximum as delignification proceeds. In view of this it is astounding, according to Ritman, that many investigators emphasize the fact that the sum of the weight of holocellulose and lignin should equal the weight of the starting material.

Sitch (19) found that some pentosan, uronic acid, and acetyl was lost when the resulting holocellulose contained 1.2% lignin. Further work on the constituents of chlorite waste liquor was done by Pearl and Wise (15, 16) who isolated 6-chlorovanillin, chlorolignins, and other materials, and by Barton (2) who separated and characterized two chlorite lignins. While the present investigation was in progress Bublitz (3) reported the presence of large quantities of carbohydrates in the waste liquor, even when relatively mild delignification conditions were used.

The information summarized above shows conclusively that the conventional method of chlorite holocellulose preparation does not result in a completely selective isolation of the carbohydrate constituents of the wood; unless some lignin is allowed to remain in the product some carbohydrate is lost. This paper reports the results of attempts to obtain complete selectivity by a different method which is based on the following suppositions:

- a. The prolonged action of chlorite solutions converts all of the lignin to dialyzable fragments.
- b. The carbohydrates which are solubilized during this treatment are substantially unaffected by the reagent and are nondialyzable.

If these suppositions are true it should be possible to carry out a selective holocellulose preparation by prolonged reaction with chlorite; the holocellulose would then consist of two fractions, i.e., the fraction insoluble in the reaction mixture and the soluble nondialyzable fraction of the waste liquors.

It was thus planned to observe the course of the reaction by conducting delignifications for various times but otherwise under similar conditions. If the above-mentioned suppositions are true the sum of the weights of the two fractions and of the lignin in the original wood will in time approach the weight of the original wood.*

EXPERIMENTAL

1. Analytical

Because of the hygroscopicity of the fractions all samples were handled and analyzed at equilibrium with the atmosphere; however, all analytical data are given in terms of oven dry material. Sodium was determined by the addition of sulphuric acid, ignition, and weighing as the sulphate; chlorine by the Parr bomb

* This argument, as well as other similar ones given in this paper, is based on the assumption that the lignin determination on the original extractive-free wood does in fact give the percentage of noncarbohydrate matter present.

method; moisture by drying at 103° overnight. Methoxyl and lignin analyses were carried out according to TAPPI Standards.

2. Raw Materials

White birch and black spruce sawdust (35–60 mesh, extractive-free according to TAPPI Standard T 12 m-45) were used. The birch sawdust contained 18.0% lignin, 0.1% ash, and 6.04% methoxyl; the spruce sawdust contained 27.0% lignin, 0.2% ash, and 4.44% methoxyl. The sodium chlorite was the analytical grade supplied by Mathieson Chemical Corporation.

3. Delignification

It was essential that the hydrolysis of the carbohydrates be kept at a minimum; consequently the delignifications were carried out at room temperature and under mildly acid conditions. Four series of experiments were carried out;

TABLE I
DELIGNIFICATIONS

Species	pH	Reaction time, weeks	Insol. fraction		Sol. fraction		Sol. fraction + insol. fraction + lignin in original wood, %*
			Na, %	Yield, %*	Na, %	Yield, %*	
Birch	4–5	2	0.9	72.0	3.0	11.6	101.6
		4	0.8	62.2	3.0	19.8	100.0
		6	0.6	59.9	3.4	20.3	98.2
Birch	6–7	1	1.0	81.8	4.7	8.1	107.5
		2	0.7	75.0	4.1	8.5	101.1
		3	0.7	70.0	3.6	12.3	99.9
		4	0.8	66.7	4.2	14.8	99.1
Spruce	4–5	1	1.1	78.8	5.0	10.1	115.9
		2	1.2	68.9	4.7	15.1	111.0
		3	1.0	62.2	5.1	20.2	109.4
		4	0.7	58.4	4.6	22.2	107.6
		5	0.8	55.5	4.6	23.6	106.1
		6	0.7	53.4	4.6	25.3	105.7
Spruce	6–7	1	1.2	78.0	4.5	9.9	114.9
		2	1.1	69.0	4.0	14.1	110.1
		3	0.7	66.2	4.1	14.8	108.0
		4	0.9	61.6	3.8	18.6	107.2
		5	0.8	56.8	4.4	20.8	104.6
		6	0.7	55.4	4.1	22.2	104.6

* Based on original wood and corrected for sodium content.

two with birch and two with spruce, with one series for each species at pH 4–5 and the other at pH 6–7. Samples of sawdust (10 gm.) were weighed accurately and placed in widemouth glass-stoppered bottles. A filtered solution of 20 gm. sodium chlorite in 120 ml. water, adjusted to the desired pH with hydrochloric acid, was added to each bottle. Each week the pH of each solution was readjusted to its original value with sodium carbonate or hydrochloric acid and the solution was reinforced by the addition of a filtered solution of 10 gm. sodium chlorite in 15 ml. water, the pH of which had been adjusted to the proper value.* From time

* Care was taken in handling the bottles because the chlorine-dioxide-containing mixture in the gaseous phase was thought to be potentially hazardous; no explosions were experienced in this work.

to time a bottle was withdrawn; the contents were transferred quantitatively to a previously washed cellophane dialysis membrane and dialyzed with distilled water until all chloride and chlorite had been removed. Titration of the diffusates showed that the concentration of chlorite in the reaction mixture was maintained roughly constant by the above-mentioned reinforcing method. The dialyzed mixture was filtered and the residue washed. The combined filtrate and washings were evaporated at room temperature; the resulting residue (soluble fraction) and the filtration residue (insoluble fraction) were equilibrated to the atmosphere, weighed, and analyzed for moisture and ash. The results are given in Table I.

These data show that under the conditions used the yield of birch holocellulose (soluble and insoluble fractions combined) closely approached the theoretical yield after about three weeks, but that the corresponding yield for spruce was still about 5% above the theoretical value after six weeks.

4. Lignin Content

It is fairly well established that the conventional methods of lignin analysis are not suitable for the determination of lignin and its breakdown products in partially delignified wood samples. This was confirmed in this investigation; all fractions gave negligible residues or none at all, even those which must necessarily contain lignin derivatives because the sum of the weights of the soluble and insoluble fractions and that of the lignin in the original wood exceeds the weight of the original wood. This is in marked contrast to the results of other investigators (6, 9, 19, 24) who obtained lignin from holocellulose prepared in comparable yield at 60–80°. It is noteworthy that none of the fractions contained appreciable amounts of ethanol-soluble matter (cf. (24)).

Three characteristics thought to be indicative of lignin were examined, viz., the methoxyl content, the chlorine content, and the ultraviolet absorption spectrum.

Löschbrandt (10) examined the ultraviolet spectra of pulp solutions in 76% sulphuric acid and encountered difficulties due to the simultaneous formation of hydroxymethylfurfural. In the present investigation it was found, however, that extracts suitable for ultraviolet spectrophotometry could be prepared with concentrated ammonia, as follows: A sample (20–100 mgm.) of the fraction was allowed to stand overnight with 5 ml. 28% ammonia. The mixture was filtered and washed; the combined filtrate and washings were diluted with water to a suitable concentration, and examined in the Beckman spectrophotometer. The filtration residue from the soluble fractions was negligible; that from the insoluble fractions was dried and analyzed for methoxyl unextracted by ammonia. The absorption curves of most samples were found to have a maximum at about 280 $m\mu$ and a minimum at about 270 $m\mu$, characteristic for lignin and its derivatives (1); the others had no clearly defined maximum and minimum but were roughly horizontal in the characteristic region. The results of the analyses of the fractions are given in Table II. The ultraviolet data are presented in terms of D/C , where D is the optical density (for 1 cm. cells) at the maximum, or, where there was no maximum, at 280 $m\mu$, and C is the concentration, expressed as the

TABLE II
ANALYSIS OF FRACTIONS

Species	pH	Reaction time, weeks	Insoluble fraction			Soluble fraction			
			CH ₃ O, %	CH ₃ O unext'd. by ammonia, % based on orig. wood	D/C	Cl, %	CH ₃ O, % based on fraction	CH ₃ O, % based on orig. wood	D/C
Birch	4-5	2	1.20	0.1	2.40		4.77	0.55	25.0
		4	0.67	0.1	1.19		3.04	0.60	13.0
		6	0.53	0.1	0.92		2.75	0.56	8.8
Birch	6-7	1	1.57	0.2	4.92	0.3	7.34	0.59	48.5
		2	1.25	0.1	2.79	0.4	4.78	0.41	25.1
		3	0.99	0.1	1.74	0.4	3.54	0.44	15.6
		4	0.81	0.1	1.10	0.1	3.34	0.49	11.5
Spruce	4-5	1	1.84	0.2	17.5	0.1	6.10	0.62	103
		2	1.23	0.3	10.0	0.2	4.47	0.68	60.2
		3	0.91	0.1	6.3	0.2	4.00	0.81	45.6
		4	0.67	0.1	3.7	0	3.79	0.84	37.4
		5	0.49	0.2	2.6		3.25	0.77	27.8
		6	0.38	0.1	1.8		3.02	0.77	26.8
Spruce	6-7	1	1.82				6.14	0.61	
		2	1.28				4.78	0.68	
		3	1.14				4.29	0.64	
		4	0.87				4.00	0.74	
		5	0.59				3.53	0.74	
		6	0.45				3.18	0.70	

number of grams of sample the extract of which is contained in 100 ml. of the solution.

Correlation diagrams for methoxyl content, ultraviolet absorption, and chlorine content are given in Figs. 1 and 2. Since no significant differences were found between samples prepared at different pH values but otherwise under similar conditions this distinction has been ignored in the preparation of Figs. 1 and 2.

Figs. 1 and 2 indicate approximately linear correlation between methoxyl content, ultraviolet absorption, and chlorine content. The spread in Fig. 2 is due

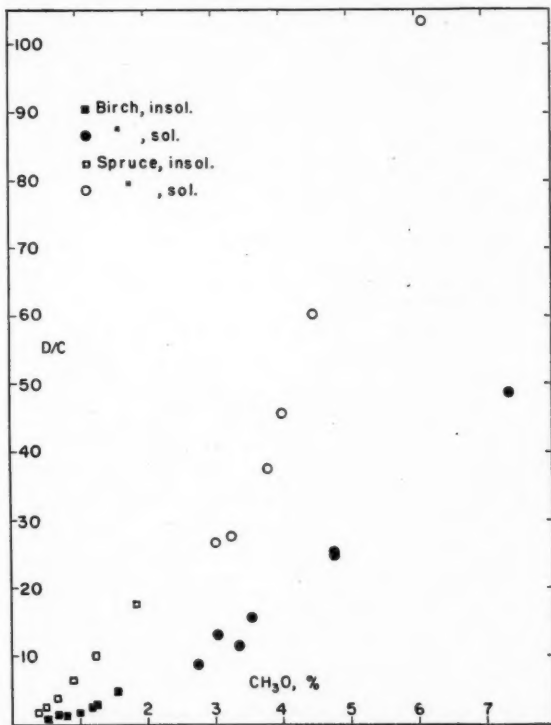


FIG. 1. Methoxyl content - ultraviolet absorption correlation diagram.

to the fact that there was considerable experimental error in the determination of the small amounts of chlorine present. The approximate proportionality of these three characteristics suggests that they all originate from the same substance, viz., a chlorinated lignin fragment which is present in varying proportions in the several fractions.

A small but comparatively constant proportion of methoxyl, based on the wood, was not extracted by ammonia from the insoluble fractions.

5. Characteristics of the Ultraviolet Absorption Curve

The effect of pH on the ultraviolet absorption curve of lignin derivatives was discussed by Ritter *et al.* (18), working with ligninsulphonic acid, and Aulin-Erdtman (1), working with acetic acid lignin, methanol lignin, and ligninsulphonic acid. Relatively little change in the position of the maximum was found; however, the absorption in the remainder of the curve increased and the minimum shifted towards longer wave lengths with increasing alkalinity.

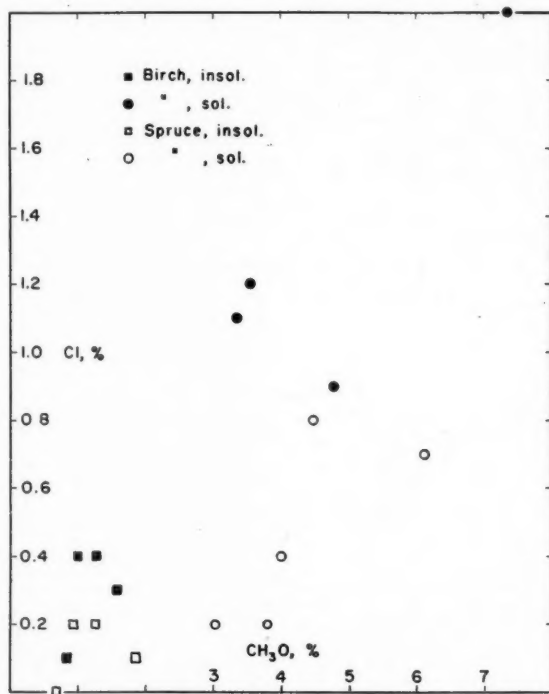


FIG. 2. Methoxyl content - chlorine content correlation diagram.

The effect of the concentration of ammonia is shown in Fig. 3. The soluble portion of birch holocellulose delignified at pH 4-5 for six weeks was used. Solution *A* was prepared by the method described above and contained about 3% ammonia; solutions *B* and *C* were diluted with ammonia solutions of appropriate strength, rather than with water, and contained 14 and 28% ammonia, respectively. The holocellulose extract concentration was the same for the three samples. The curves in Fig. 3 are similar to those given by Aulin-Erdtman (1) for ligninsulphonic acid.

The effect of acidification is shown in Fig. 4 for which the insoluble fraction of birch holocellulose delignified at pH 6-7 for two weeks was used. The alkaline solution was prepared by the method described above; the acid solution was

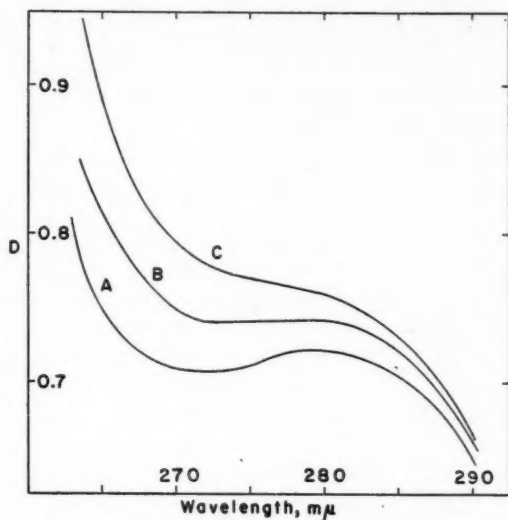


FIG. 3. The effect of ammonia concentration on the ultraviolet absorption curve.

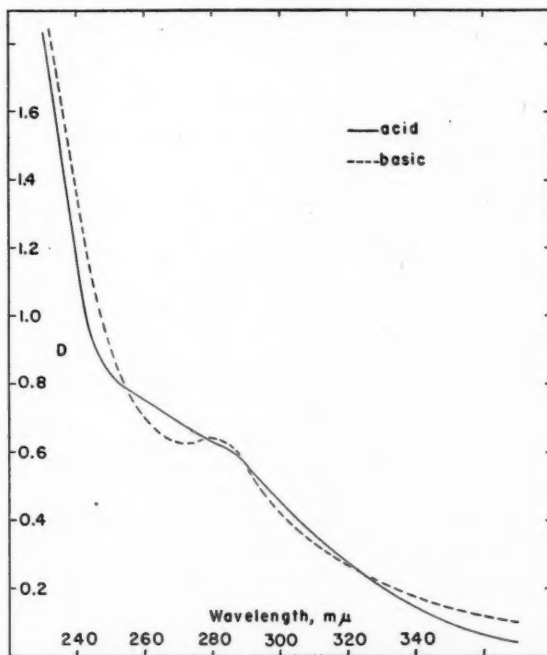


FIG. 4. The effect of acidification on the ultraviolet absorption curve.

prepared by acidification of the alkaline solution with hydrochloric acid and was diluted to the same extract concentration. Fig. 4 shows that the maximum and minimum disappear on acidification; in view of this it is perhaps not surprising that Barton (2) did not obtain maxima and minima for his chlorite lignins as the preparations used were decidedly acidic.

6. A Methoxyl-containing Substance Resistant to Chlorite Solutions

Table II shows that the methoxyl content of the soluble fractions, based on the original wood, remains roughly constant throughout the delignification. This suggests that a methoxyl-containing macromolecular substance resistant to chlorite solution is present in the soluble fractions. To confirm the presence of this substance chlorite waste liquors were subjected to the continued action of chlorite in the absence of wood. Since duplicate experiments gave essentially similar results only one will be described.

A filtered solution of 120 gm. sodium chlorite in 720 ml. water was adjusted to pH 6.1 with hydrochloric acid and mixed with 60 gm. extractive-free birch sawdust. The mixture was allowed to stand at room temperature in a loosely covered flask for 11 days. It was then filtered without washing; the filtrate was placed in a glass-stoppered graduated cylinder. Each week an aliquot of the solution was withdrawn, dialyzed, and further treated in the same manner as the soluble fractions described above. The remaining solution was adjusted to pH 6-7 and reinforced by the addition of a filtered solution of 60 gm. sodium chlorite in 90 ml. water, also adjusted to pH 6-7. The results are given in Table III. The nonvolatile content of the dialyzed sample withdrawn after the first week was 3.86 gm. per liter.

TABLE III
ACTION OF CHLORITE SOLUTION ON CHLORITE WASTE LIQUOR

Reaction time, weeks	Yield, %*	Na, %	CH ₃ O, %	Total CH ₃ O in sample, %*
1	100	4.1	6.25	100
2	98	3.2	6.12	96
3	114	5.2	5.65	103
4	112	6.9	5.38	96
5	105	6.0	5.62	95

* Based on the yield of nondialyzable material and methoxyl obtained after one week of chlorite treatment.

The results given in Table III confirm the presence of a methoxyl-containing macromolecular substance resistant to sodium chlorite. In view of the findings in Section 4, this is very likely a lignin fragment.

It is noteworthy that the yield of some fractions is rather high and that their sodium contents are noticeably higher than those of the soluble fractions given in Table I. No explanation of these results is apparent.

DISCUSSION

If demethoxylation of lignin does not occur during the reaction, the weight of lignin fragments present in any fraction may be estimated from the methoxyl

content of the fraction and that of the lignin in the original wood (calculated from the methoxyl and lignin content of the wood); if demethoxylation does occur the lignin content thus calculated is a minimum value. This "minimum lignin content" is about 1.5%* for the soluble fraction of birch holocellulose; the corresponding value for spruce is about 4%. The minimum lignin content of the insoluble fractions during the later stages of delignification is about 1–1.5% for both species. The following conclusions may thus be drawn regarding the course of the delignification:

1. For birch, the lignin fragments remaining in both fractions during the later stages of delignification amount to at least 2.5–3%; since the yield of both fractions combined is 98–99% of theoretical (original wood – lignin) at least 3.5–5% of the carbohydrates are lost. Therefore the lignin in birch is not completely converted to dialyzable fragments by the action of chlorite, and the carbohydrates are not completely retained in the insoluble and soluble nondialyzable holocellulose fractions. Thus suppositions *a* and *b*, mentioned in the introduction, are not rigidly applicable.

2. For spruce, the lignin fragments remaining in both fractions during the later stages of delignification amount to at least 5–5.5%. Therefore the lignin in spruce is not converted completely to dialyzable fragments, and supposition *a* does not apply. If the minimum lignin content can be considered as the actual lignin content, the yield of both fractions combined, corrected for their lignin content, is very close to theoretical (99–100%). Under these circumstances all the carbohydrates are retained and supposition *b* applies.

3. As delignification proceeds, the soluble nondialyzable fraction increases at the expense of the insoluble fraction.

The nature of the chlorite resistant lignin fragment is of particular interest. Wacek and Schroth (20, 21, 22) recently reported the discovery and isolation from holocellulose of a "protolignin I" which is not recovered as a solid in the lignin determination and which is resistant to chlorite solutions. It is possible that protolignin I is identical with or related to the chlorite resistant lignin fragment discussed above, the latter having been solubilized by prolonged reaction. If this were true the material balances given above would apply no longer, since protolignin I is not included in the results from the lignin determination on the original wood; the birch holocellulose would have been recovered in roughly quantitative yield but the excess yield of spruce holocellulose would be difficult to explain.

ACKNOWLEDGMENT

Financial support given by the Research Council of Ontario for this work is gratefully acknowledged.

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SOLVENT EFFECTS IN CIS-TRANS ISOMERIZATION¹

By P. LUNER² AND C. A. WINKLER

ABSTRACT

The *cis-trans* isomerizations of azoxybenzene, *p,p'*-azoxytoluene, and *p*-chlorobenzenediazocyanide have been investigated in a variety of solvents. While linear relations between $\log k$ and $(P_S')^{\frac{1}{2}}$ were found to exist for reactions of the azoxy compounds in solvents of internal pressure greater than nine, no such correlation was found for the diazocyanide. Linear relations have been observed between the logarithms of the rate constants for isomerization of azobenzene, azoxybenzene, and *p,p'*-azoxytoluene in a series of solvents.

INTRODUCTION

According to relations which may be derived from existing theories of solutions and of chemical kinetics (5), both E (activation energy) and $\log k$ (k = rate constant) for a nonionic unimolecular reaction in different solvents should be linearly related to $(P_S')^{\frac{1}{2}}$ (P_S' = internal pressure of solvent). The signs of the slopes in these relations are determined by the relative magnitudes of the internal pressures of the reactants (P_X) and activated complex (P_M^*). The equation relating E and $(P_S')^{\frac{1}{2}}$ was found to hold for the isomerization of azobenzene in several solvents, but a plot of $\log k$ against $(P_S')^{\frac{1}{2}}$ showed deviations for the reaction in highly polar solvents.

To examine further the effect of solvent in such reactions, studies have now been made of the *cis-trans* isomerizations of azoxybenzene and of *p,p'*-azoxytoluene in several solvents. Also, the solvent effect on the rate of isomerization of *p*-chlorobenzenediazocyanide, studied elsewhere by measurements of dielectric constants of the solutions (8), was reinvestigated using a spectrophotometric analysis with the intent to include a wider range of internal pressures of the solvents used.

EXPERIMENTAL AND RESULTS

The *cis* and *trans* isomers of azoxybenzene and *p,p'*-azoxytoluene were prepared according to the procedures of Reissert (12) and Müller and Kreutzman (11) respectively. Separation of the two isomers was possible in both cases by taking advantage of the greater solubility of the *trans* form in petroleum ether.

Characterization of the azoxy isomers by their ultraviolet absorption spectra, using a Beckman Model "DU" Spectrophotometer, gave results that agreed with those of Müller and co-workers (9, 10), from which it was apparent that the concentrations of the isomers could be accurately determined with the spectrophotometer at wave lengths of 3300 Å (azoxybenzene) and 3350 Å (*p,p'*-azoxytoluene). Owing to the relatively rapid isomerization of the *cis* forms in some of the solvents, no attempt was made to establish calibration curves as in the previous study (5). Instead, first order rate constants were obtained from the

¹ Manuscript received April 2, 1952.

Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Que., with financial assistance from the National Research Council of Canada.

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slope of a plot of $\log (D_0 - D_t)$ against time, in accordance with the expression

$$k = \frac{2.303 \log (D_0 - D_t)}{t}$$

where k is the specific rate constant,

D_0 is the optical density of the solution ($\times 10^3$) after isomerization is complete,

D_t is the optical density of the solution ($\times 10^3$) at time t , and

t is the time interval corresponding to the change in optical density, $(D_t - D_0)$.

Experiments with two different preparations of *cis*-azoxybenzene gave concordant first order constants, which indicated that if there were impurities in the preparations they did not alter significantly the optical densities of the solutions. Moreover, after isomerization was complete, the solutions gave the same absorption spectra as a solution of the pure *trans* isomer in methanol.

The *cis* and *trans* isomers of *p*-chlorobenzenediazocyanide were prepared according to the procedure of Anderson, Bedwell, and LeFevre (1). The absorption spectra for a series of substituted benzenediazocyanides have previously been determined by LeFevre and Wilson (8). They observed a strong absorption band for *p*-chlorobenzenediazocyanide at 4500 Å, with the *cis* isomer absorbing more strongly than the *trans* in this region.

Reproducible first order rate constants were obtained in the manner indicated for the azoxy compounds.

The solvents used were supplied by Brickman and Co., Montreal. They were dried with appropriate desiccants, distilled, and the middle fractions with correct boiling points collected. Approximately 15 ml. of solutions of the *cis* isomer were placed in ½-oz. amber bottles, tightly closed with aluminum gaskets and screw caps, and placed in conventional thermostats controlled to $\pm 0.05^\circ\text{C}$. After the desired reaction times the bottles were removed, the contents cooled rapidly, and the optical density determined, using the pure solvent as a blank. The isomerization of *p*-chlorobenzenediazocyanide was studied in a darkened room to avoid errors due to photochemical isomerization (6).

With heptane and cyclohexane as solvents the reaction rate of the azoxy compounds at room temperature was appreciable, and the error with these solvents is somewhat larger than with the others in which reaction during the time required for optical analysis was insignificant.

The kinetic data for the isomerization of azoxybenzene, *p,p'*-azoxytoluene, and *p*-chlorobenzenediazocyanide are summarized in Tables I, II, and III. An indication of the accuracy of the work was obtained by calculating the mean deviation of the temperature coefficients for successive temperature intervals from the mean value ($E_{\text{calc.}}$) in each solvent. The activation energies determined from Arrhenius plots are also recorded. The activation energies for the isomerization of *p*-chlorobenzenediazocyanide are estimated to be accurate to ± 125 calories.

TABLE I
ISOMERIZATION OF AZOXYBENZENE IN VARIOUS SOLVENTS
SUMMARY OF KINETIC DATA

Solvent	Temp., °C.	Rate, min. ⁻¹	log <i>k</i>	<i>E</i> _{calc.} , kcal./mole	Mean dev. of temp. coeff. from <i>E</i> _{calc.} , kcal./mole	<i>E</i> _{graphical}	log <i>A</i>
Methanol	58.68	0.0295	2.470	22.08	0.16	22.25	13.05
	48.85	0.00777	3.890				
	29.50	0.00115	3.060				
Ethanol	59.3	0.0550	2.741	22.89	0.57	22.9	13.86
	58.68	0.0526	2.721				
	46.04	0.0130	2.114				
	45.92	0.01442	2.160				
	45.75	0.0123	2.090				
	29.93	0.00185	3.267				
	29.50	0.00186	3.269				
Heptane	35.35	0.0452	2.665	22.40	0.51	22.4	14.58
	29.50	0.0228	2.358				
	25.03	0.0131	2.117				
	14.49	0.00359	3.556				
Acetonitrile	59.3	0.0460	2.663	24.27	0.12	24.3	14.82
	58.68	0.0422	2.626				
	58.58	0.0420	2.624				
	45.92	0.00962	3.982				
	29.93	0.001265	3.102				
	29.50	0.00123	3.090				
Butanol	46.02	0.02053	2.312	22.81	0.86	23.2	14.00
	45.94	0.0221	2.344				
	45.75	0.0214	2.330				
	29.93	0.00301	3.478				
	29.50	0.003035	3.482				
	25.03	0.001735	3.239				
Hexanol	45.97	0.0285	2.455	22.8	0.37	22.7	14.11
	35.35	0.00789	3.897				
	25.03	0.00222	3.346				
Benzene	46.00	0.0489	2.689	22.22	0.48	22.8	13.93
	35.35	0.01345	2.129				
	29.50	0.00662	3.821				
	25.03	0.003875	3.583				
Toluene	45.94	0.0583	2.766	23.29	0.49	22.8	14.78
	45.75	0.0613	2.787				
	35.33	0.01695	2.229				
	25.03	0.00460	3.663				
Cyclohexane	35.35	0.0433	2.637	22.35	0.57	22.4	14.63
	29.50	0.0219	2.340				
	15.49	0.00351	3.545				

TABLE II
ISOMERIZATION OF *p,p'*-AZOXYTOLUENE IN VARIOUS SOLVENTS
SUMMARY OF KINETIC DATA

Solvent	Temp., °C.	Rate, min. ⁻¹	log <i>k</i>	<i>E</i> _{calc.} , kcal./mole	Mean dev. of temp. coeff. from <i>E</i> _{calc.} , kcal./mole	<i>E</i> _{graphical} ■	log <i>A</i>
Methanol	59.0	0.0173	2.238	23.88	0.29	23.8	14.03
	45.85	0.004085	3.611				
	29.50	0.000524	4.719				
Ethanol	58.65	0.0294	2.468	23.21	0.87	23.4	13.84
	45.95	0.00777	3.891				
	45.85	0.00756	3.879				
	29.50	0.000980	4.991				
Acetonitrile	58.65	0.0260	2.415	24.12	0.17	24.1	14.36
	45.85	0.00601	3.779				
	35.35	0.001655	3.219				
	29.50	0.000759	4.880				
Hexanol	45.97	0.0167	2.223	23.85	0.17	23.5	14.60
	35.35	0.00442	3.648				
	25.03	0.00116	3.064				
Benzene	45.85	0.0241	2.381	23.44	0.10	23.4	14.50
	36.13	0.00753	3.877				
	29.53	0.00320	3.505				
	25.03	0.00178	3.251				
Toluene	45.85	0.0308	2.489	23.68	0.48	23.6	14.75
	36.13	0.00967	3.985				
	29.52	0.00394	3.596				
	25.03	0.00223	3.349				
Butanol	46.04	0.0118	2.072	23.22	0.57	23.1	14.01
	45.85	0.0119	2.076				
	35.35	0.00323	3.509				
	29.50	0.00155	3.190				
	25.03	0.000875	4.942				
Cyclohexane	35.31	0.0256	2.408	22.12	0.41	22.9	14.14
	25.03	0.007495	3.875				
	14.49	0.002155	3.333				
Heptane	35.35	0.0273	2.436	22.67	0.66	22.9	14.54
	25.03	0.00792	3.899				
	15.49	0.002162	3.335				

TABLE III
ISOMERIZATION OF *p*-CHLOROBENZENEDIAZOCYANIDE IN VARIOUS SOLVENTS
SUMMARY OF KINETIC DATA

Solvent	Temp., °C.	Rate, min. ⁻¹	log <i>k</i>	<i>E</i> _{calc.} , kcal./mole	log <i>A</i>
Heptane	29.93	0.0008831	4.946	22.40	13.12
	45.80	0.005346	3.728		
	58.57	0.02244	2.351		
Cyclohexane	29.93	0.0007816	4.893	22.51	13.18
	45.80	0.005284	3.723		
	58.57	0.02061	2.314		
Benzene	29.93	0.001656	3.219	22.92	13.77
	45.80	0.01091	2.038		
	58.57	0.04656	2.668		
Chlorobenzene	29.93	0.002432	3.386	21.92	13.23
	45.80	0.01517	2.181		
	58.57	0.005754	2.760		
<i>o</i> -Dichlorobenzene	29.93	0.003055	3.485	21.80	13.25
	45.80	0.01879	2.274		
	58.57	0.07516	2.876		
Chloroform	29.93	0.004560	3.659	20.05	12.18
	45.80	0.02438	2.387		
	58.57	0.08453	2.927		

DISCUSSION

When the *cis* isomer of *p*-chlorobenzenediazocyanide was dissolved in cyclohexanone, pyridine, or nitromethane, the changes in optical density of the solutions at 4500 Å were so rapid that measurements could not be made. After several minutes these solutions became highly colored. Apparently the *cis* isomer suffered decomposition in these solvents. On the other hand, the absorption spectra of *trans*-*p*-chlorobenzenediazocyanide in cyclohexanone and pyridine showed the characteristic absorption maxima at 4500 Å and no strong color formation occurred, indicating that the *trans* isomer was stable in the same solvents. Solutions of the *cis* isomer (0.08 gm. per liter) in cyclohexane, chlorobenzene, *o*-dichlorobenzene, heptane, or chloroform, were all of approximately the same optical densities and no strong color formation occurred. The same was true of the *trans* isomers in these solvents. Hence in these solvents both isomers were stable. It would appear then, that the *cis* isomer but not the *trans* isomer decomposes in ionizing solvents, while both forms are stable in nonionizing solvents.

Stephenson and Waters (13) in studying the decomposition of several *cis* and *trans* derivatives of benzenediazocyanide in a variety of solvents have reached a similar conclusion.

A comparison of the data of LeFevre and Northcott (7) for the isomerization of *p*-chlorobenzenediazocyanide with those in Table III reveals that not only are there serious discrepancies between the relative rates in the same series of solvents but rate data for the isomerization reaction in pyridine and cyclohexanone could

not be obtained at all by spectrophotometric analysis. It appears therefore that measuring the rates of isomerization of this compound by following the changes in the dielectric constants in these solvents may not be an entirely reliable method. Unfortunately, owing to the limited type of solvents that could be used, it was not found possible to extend the range of internal pressure values significantly, to test the relations between E and $(P_S^\dagger)^{\frac{1}{2}}$ and $\log k$ and $(P_S^\dagger)^{\frac{1}{2}}$.

The internal pressures of the solvents used with the azoxy compounds were calculated from $P = \Delta E/V$, where ΔE is the energy of vaporization and V the molar volume of the liquid. Values of ΔE were calculated (using $\Delta E = \Delta H - RT$) from values of ΔH taken from the International Critical Tables and the Landolt-Bornstein Tables, or estimated from vapor pressure data.

Unfortunately, any possible correlation between $(P_S^\dagger)^{\frac{1}{2}}$ and E or $\log A$ in the isomerization of the azoxy compounds is obscured by the small range in these quantities relative to the error. It might be possible to reduce the error in the activation energies by studying the reactions at lower temperatures with the aid of a spectrophotometer fitted with a low-temperature thermostat.

The large variation in k , with small relative error, appears to permit a reasonably good linear relation between $\log k$ and $(P_S^\dagger)^{\frac{1}{2}}$ (Fig. 1) for each of the isomer-

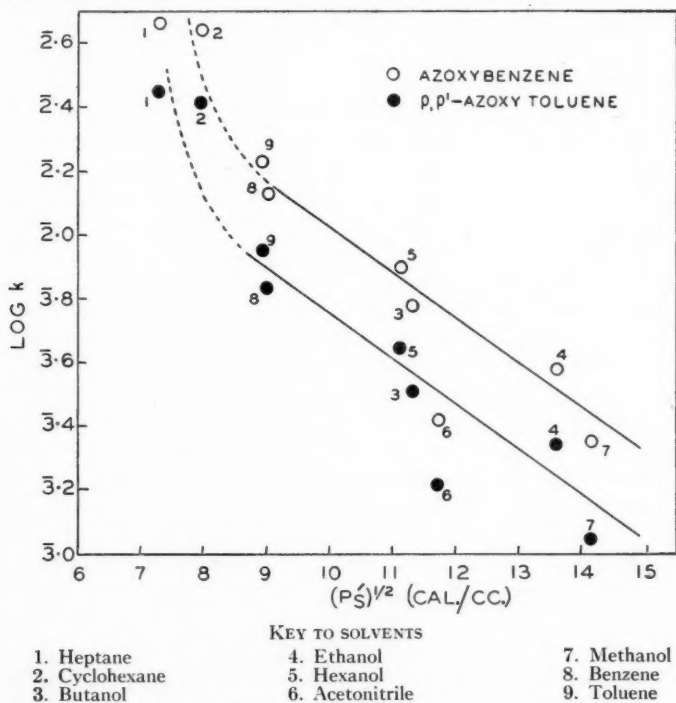


FIG. 1. Relation between $\log k$ and $(P_S^\dagger)^{\frac{1}{2}}$ at 35°C. for the isomerization of azoxybenzene and of p,p' -azoxytoluene in different solvents.

ization reactions in solvents with internal pressure greater than about nine. At lower internal pressures, however, there is a sharp increase in k as the internal pressure is further decreased, which might mark a transition from polar to non-polar solvents. It is interesting, perhaps, that in the highly polar solvent, acetonitrile, the value of k appears to be out of line in the opposite sense, in a manner similar to that observed in the isomerization of azobenzene (5).

The slopes of the lines in Fig. 1 are negative. Reference to the equation relating the rate and the internal pressure of the solvent (5) indicates that the internal pressures of the reactants are greater than those of the corresponding activated complexes. These results might be anticipated by a consideration of the dipole moments of the reactants and activated complex since in many instances there is a close parallel between dipole moment and internal pressure (3).

In a given solvent, the rate of isomerization of azoxybenzene is about 50 times that of azobenzene at the same temperature. This might be explained by the tendency for the oxygen atom in the former to take up electrons from the $N = N$ bond to form a coordinate covalent link with the nitrogen to which it is attached, and thus reduce the double bond character of the $N = N$ bond and facilitate isomerization. The inductive effect of the methyl group in p,p' -azoxytoluene is probably responsible for the smaller rate of its isomerization in comparison with azoxybenzene.

According to the treatment referred to previously (5), the effect of solvent on

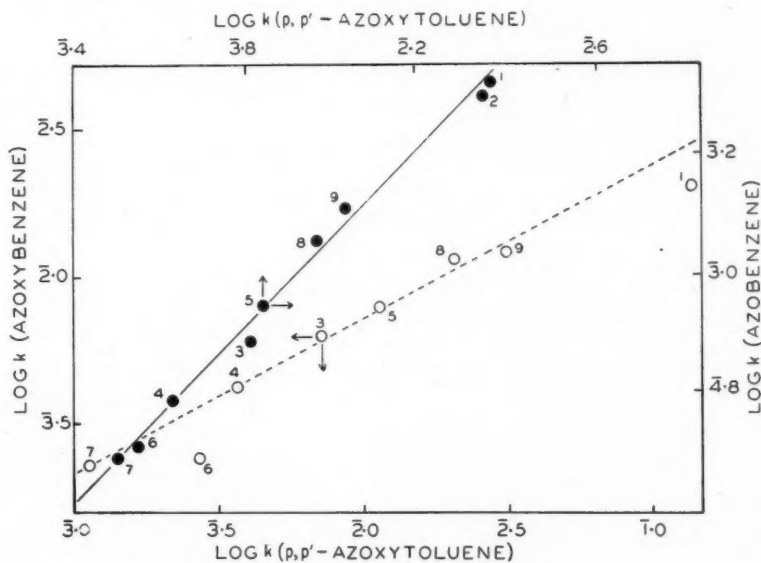


FIG. 2. Relations between $\log k$ values for the isomerizations of azoxybenzene and p,p' -azoxytoluene at 35.35°C., and for the isomerizations of azobenzene and p,p' -azoxytoluene at 43.85°C., in different solvents.

the rate of isomerization of azobenzene, azoxybenzene, and *p,p'*-azoxytoluene can be expressed by equations of the type,

$$\log k = J - L(P_S')^{\frac{1}{2}}$$

where *J* and *L* are characteristic of a given reaction at a given temperature. It follows that at a given temperature the ratio of the rates for any two reactions in a series of solvents should be constant. The linear relations shown in Fig. 2 provide rather striking evidence in support of this conclusion.

Analogous relations have been observed to relate the influence of a series of substituents on the rate of a given type of reaction to the influence of the same substituents on the rate of a related process (2). These relations have been interpreted by Evans and Polanyi (4) to result from a constant ratio of the free energies of activation of the two processes, in accordance with

$$\log k = \text{const.} - \frac{\Delta F^*}{2.303RT}$$

where ΔF^* is the free energy of activation. A similar interpretation of the relations in Fig. 2 appears to be reasonable.

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STUDIES ON THE FORMATION OF HEXAMINE¹

BY T. R. INGRAHAM² AND C. A. WINKLER

ABSTRACT

Rate curves have been determined for the reaction of ammonium nitrate with formaldehyde in glacial acetic acid solution at 25°C., 35°C., 45°C., and 55°C. over a range of initial mole ratios (formaldehyde : ammonia) of 0.75:1 to 9.0:1. Data obtained at 25°C. show a definite induction period in the formation of hexamine. The length of the induction period is not changed by increasing ammonium nitrate concentrations above the theoretical (1.5:1), but may be appreciably shortened by initial additions of excess formaldehyde or of sodium acetate. From 35°C. upward, the induction period is not apparent. The order of the reaction with respect to formaldehyde has been determined from initial rate data, and an activation energy calculated. The reactions in general appear analogous to those found in slightly acid aqueous systems.

INTRODUCTION

In a previous paper from this laboratory (5) it was shown that formaldehyde and ammonium nitrate reacted in glacial acetic acid to form hexamine dinitrate. A more detailed study of this reaction in this medium has now been made, to enable comparison with the analogous reactions in aqueous systems (3).

EXPERIMENTAL

Reagents

Redistilled glacial acetic acid (m.p. 16.60°C.) was used as solvent. Formaldehyde solutions were prepared by depolymerization of paraformaldehyde in hot (70°C.) glacial acetic acid, and standardized volumetrically by determining the extent of reduction in a Nessler solution (2).

Ammonium nitrate, ammonium acetate, and sodium acetate solutions in glacial acetic acid were prepared by weight, using anhydrous reagent grade salts.

Nitric acid of 99.6% purity was prepared by distilling the commercial product (68%) with twice its volume of concentrated sulphuric acid, after which the oxides of nitrogen were removed from the distillate with a stream of dry nitrogen. The acid was stored at - 68°C.

Analytical Methods

The hexamine content of a partially reacted formaldehyde - ammonium salt solution in glacial acetic acid was determined by adding a cool slurry of mercuric chloride in aqueous sodium hydroxide. The sodium hydroxide content was adjusted so that the pH of the mixture was brought to 4.0 during precipitation of the hexamine - mercuric chloride complex from an appropriate aliquot of the reaction mixture. Under these conditions, estimation of hexamine from the dry weight of the precipitate was found to be quantitative within 0.2% of the theoretical, for solutions containing in excess of 50 mgm. per liter of hexamine. The composition of the complex was constant, with a gravimetric factor of 0.0768.

Ammonia was determined on the filtrate following the removal of the hex-

¹ Manuscript received April 2, 1952.

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amine - mercuric chloride complex, by adding an excess of sodium sulphide and subjecting the mixture to a Kjeldahl distillation. No interference of nitrate ion or strong reducing agents was observed.

Formaldehyde was also determined on the filtrate from the hexamine precipitation. Precipitation with dimedon (7) after buffering the solution at pH 6.0 and converting the mercuric ion to the iodide complex, HgI_4^- , gave very satisfactory results. Alternatively, the formaldehyde was expelled from the filtrate by boiling it with dilute sulphuric acid. The distillate was received in Nessler's reagent, and titrated with standard iodine and thiosulphate solutions to determine the extent of the reduction. Both the gravimetric and volumetric methods gave essentially the same results.

Reactions were allowed to proceed in stoppered flasks immersed in suitable thermostats, controlled to $\pm 0.02^\circ\text{C}$. From time to time, aliquots were withdrawn, and the reaction quenched by addition of a mercuric chloride - sodium hydroxide slurry. Complex formation between the mercuric ion and ammonia prevented further reaction during the precipitation of the hexamine complex. All reaction mixtures were 0.025 molar in ammonium nitrate, while the concentration of formaldehyde was varied.

RESULTS AND DISCUSSION

Experiments were made over the temperature range $25\text{--}55^\circ\text{C}$., using six different molar ratios of formaldehyde to ammonium nitrate ranging from 0.75:1.0 to 9.0:1.0. Calculations of the theoretical hexamine yield were based on the overall reaction,



At 25°C . a definite induction period in the rate of hexamine formation was noted. Increasing the ratio of formaldehyde to ammonium nitrate beyond the theoretical 1.5:1.0 did not appreciably alter the length of the induction period but did increase the rate of hexamine formation after the induction period had passed (Fig. 1). Decreasing the formaldehyde: ammonium nitrate ratio below the theoretical appreciably shortened the induction period, and it was found that it could be almost completely eliminated by the initial addition of small amounts of sodium acetate to the reaction mixture. If sodium acetate additions were delayed until the induction period was over, the rate of hexamine formation increased rapidly after the addition was made. If additions of excess formaldehyde were made to the reaction mixture after the induction period was over, the formation of additional hexamine was prevented (Fig. 1).

Experiments failed to show any indication of an induction period at 35°C . However, if ratios of formaldehyde to ammonium nitrate greater or less than the theoretical (1.5:1.0) were used, discrepancies were observed between rate curves based on hexamine formation and on disappearance of formaldehyde and ammonia (Fig. 2). By arguments similar to those advanced in earlier studies with aqueous systems (3), it seems reasonable to conclude that with excess formaldehyde or excess ammonium nitrate, the hexamine precipitate is contaminated with a by-product or intermediate.

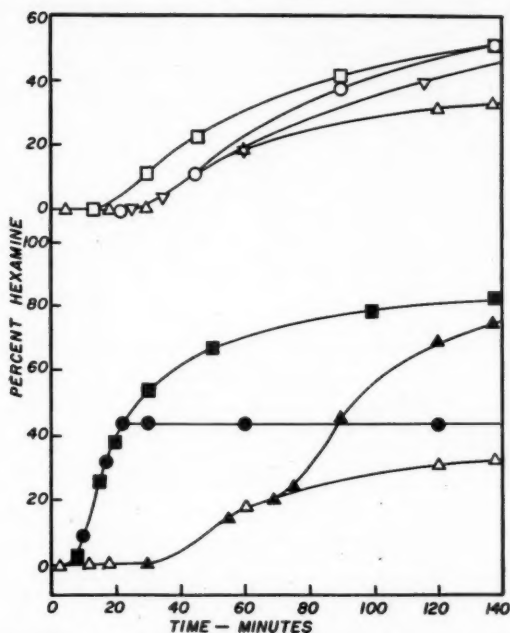


FIG. 1. Rate curves for reactions at 25°C. Initial ammonium nitrate concentration 0.025 *M*. Initial ratio formaldehyde: ammonium nitrate;

Top curves Δ 1.5 :1.0 (theoretical)

∇ 3.0 :1.0

\circ 6.0 :1.0

\square 0.75:1.0

Bottom curves Δ 1.5 :1.0 (theoretical)

\blacktriangle 1.5 :1.0 plus a 5 *M* excess of sodium acetate (relative to initial ammonium nitrate) added after 70 min.

\blacksquare 1.5 :1.0 plus an initial 10 *M* excess of sodium acetate (relative to initial ammonium nitrate)

\bullet 1.5 :1.0 plus an initial 10 *M* excess of sodium acetate (relative to initial ammonium nitrate) to which a 5 *M* excess formaldehyde (relative to initial ammonium nitrate) was added after 20 min.

The maximum in the curve for recoverable hexamine (Fig. 2) in the presence of excess formaldehyde indicated that decomposition of hexamine proceeded concurrently with its formation. This decomposition was studied further at 35°C. using reaction mixtures of formaldehyde and ammonium nitrate ranging through eight different molar ratios from 0.38:1.0 to 9.0:1.0, the solutions being analyzed for hexamine after $\frac{1}{2}$, 2, 6, and 24 hr. reaction time. The results, shown in Fig. 3, indicate that decomposition of hexamine is not a serious complication during the earlier stages of its formation, but becomes appreciable before the lapse of six hours. Study of the same reaction at 50°C. showed a marked acceleration of both the reaction to form hexamine, and the reaction bringing about its decomposition; the latter assumed significant proportions at this temperature after one-half hour at molar ratios of formaldehyde to ammonium nitrate greater than about 4:1.

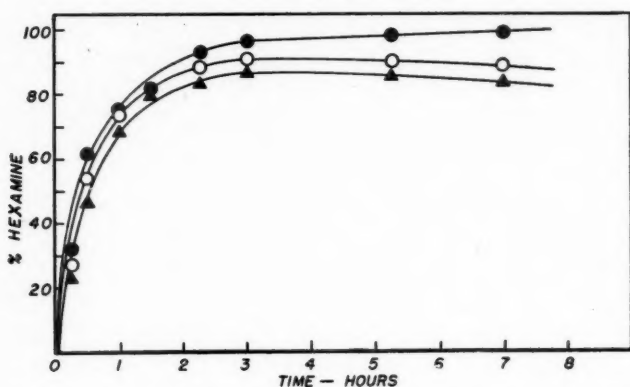


FIG. 2. Rate curves at 35°C. Initial ammonium nitrate concentration 0.025 *M*. Initial formaldehyde: ammonium nitrate ratio 6.0:1.0.

- Based on formaldehyde disappeared.
- Based on material precipitated by mercuric chloride.
- ▲ Based on ammonia disappeared.

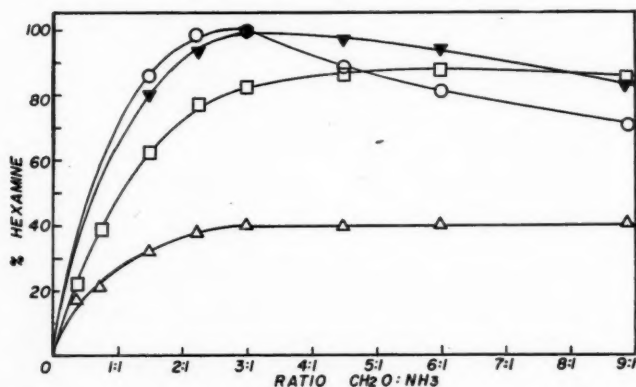


FIG. 3. Hexamine recovery at 35°C. as a function of initial mole ratio of formaldehyde: ammonium nitrate. Initial ammonium nitrate concentration 0.025 *M*.

- △ After 1/2 hr. reaction time.
- After 2 hr. reaction time.
- ▼ After 6 hr. reaction time.
- After 24 hr. reaction time.

By a rapid decomposition of pure hexamine with excess formaldehyde at the reflux temperature of glacial acetic acid, a mixture of products, with a strong amine odor, was obtained. One of these products was isolated as a picrate and identified as 1,5-endomethylene 3,7-dimethyl 1,3,5,7-tetrazocyclooctane (m.p. 195–196°C.) This substance has also been prepared by the action of excess aqueous formaldehyde on hexamine picrate in alcoholic solution (6).

Since nitric acid is formed during the reaction of formaldehyde with ammonium nitrate, it seemed possible that its presence might be responsible for at

least part of the observed destruction of hexamine. The reaction between formaldehyde and ammonium acetate was therefore studied at 35°C. in glacial acetic acid to which had been added 99.6% nitric acid in the molar ratio of 10:1 with the ammonium acetate present. The results are shown in Fig. 4, from which

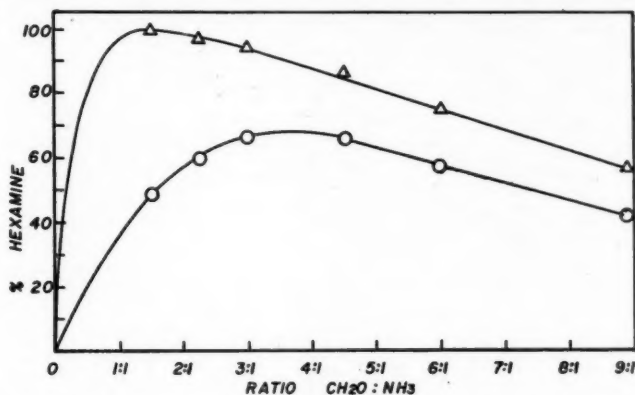


FIG. 4. Hexamine recovery after 24 hr. reaction time at 35°C. as a function of initial molar ratios of formaldehyde to ammonium acetate. Initial ammonium acetate concentration 0.025 *M*.
 Δ In the absence of nitric acid (added).
 ○ In the presence of an initial 10 *M* excess of 99.6% nitric acid (relative to initial ammonium acetate).

it is evident that the decrease in hexamine yield with increasing molar ratios of formaldehyde to ammonium acetate beyond the optimum was essentially parallel in the two systems. This indicates that destruction of hexamine was not accentuated by the presence of nitric acid. The figure shows also that, in the presence of nitric acid, the optimum ratio of formaldehyde to ammonium acetate was shifted from 1.5:1.0 to 3.7:1.0 and the maximum yield of hexamine obtainable was considerably reduced, when nitric acid was present. These two observations might be interpreted to indicate destruction of formaldehyde in the presence of nitric acid. However, when the rate of hexamine formation was compared with the rate of formaldehyde disappearance in formaldehyde-ammonium acetate (1.5:1.0) reaction mixtures in glacial acetic acid containing various amounts of nitric acid, the results shown in Fig. 5 were obtained. These results indicate that formaldehyde disappearance was due only to hexamine formation, and that the main effect of nitric acid was to reduce the rate of the reaction by which hexamine is produced. A slower consumption of formaldehyde when nitric acid is present would presumably favor destruction of some hexamine by unreacted formaldehyde at low molar ratios of formaldehyde to ammonium acetate, and this destruction would, in turn, consume formaldehyde which would otherwise be available for hexamine formation. In this way, the decrease in maximum yield of hexamine and the increase in optimum molar ratio of formaldehyde to ammonium acetate shown in Fig. 4 might be explained.

It seemed possible, even likely, that nitric acid reduced the rate of hexamine

formation from formaldehyde and ammonium acetate in glacial acetic acid by bringing about a reduction in free ammonia content of the solution. Displace-

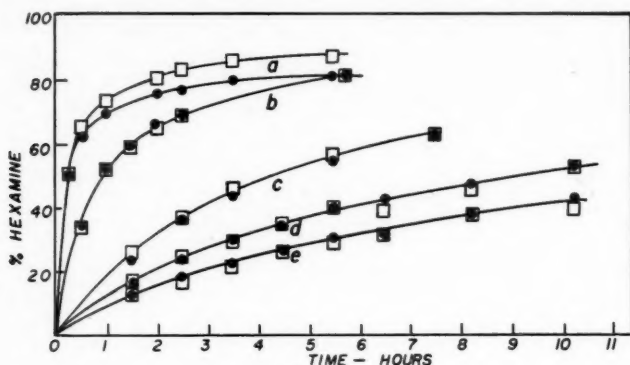


FIG. 5. Rate curves at 35°C.

□ Based on material precipitated by mercuric chloride.

● Based on formaldehyde recovered.

Initial ammonium acetate concentration 0.025 *M*.

Initial molar ratio formaldehyde : ammonium acetate : nitric acid.

a. 1.5 : 1.0 : 0.5

b. 1.5 : 1.0 : 1.0

c. 1.5 : 1.0 : 2.0

d. 1.5 : 1.0 : 4.0

e. 1.5 : 1.0 : 6.0

ment to the left, by formation of NH_4^+ ion, of the equilibrium



would account for the observed retardation if hexamine formation were dependent upon reaction of formaldehyde with ammonia rather than the ammonium ion. Evidence for the existence of such an equilibrium is found in the fact that the distillate from a concentrated solution of ammonium acetate in acetic acid contains appreciable amounts of ammonia (4).

Owing probably to the existence of the above equilibria, of which the constants are not known at present, it has been impossible to correlate the rate of hexamine formation with the concentration of ammonium nitrate, ammonium acetate, or sodium acetate in the system, except in a qualitative way. However, in studies of the early stages of the reaction to form hexamine over the temperature range 35–55°C., initial rates, obtained by tangents to the rate curves at the origin, indicate the reaction to be first order with respect to formaldehyde, with an activation energy, deduced from a good Arrhenius line, of 20.5 kcal. This value compares favorably with the value of 19.5 kcal. obtained by Baur and Rüetschi for the reaction of formaldehyde with ammonia in aqueous solution (1).

In general, it would appear from the observations made on the present system that it is fundamentally similar to the analogous reactions in a slightly acid aqueous system.

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THE REACTIONS OF TITANIUM TETRAIODIDE ON HEATED TITANIUM AND TUNGSTEN SURFACES¹

BY T. R. INGRAHAM² AND L. M. PIDGEON³

ABSTRACT

When TiI_4 is passed over a hot bar of Ti metal, the reactions observed depend on whether the temperature of the bar is above or below $1100^\circ C$. If the temperature is below $1100^\circ C$, Ti is rapidly etched from the bar to form TiI_2 , which through further reaction with TiI_4 is converted to TiI_3 . A mixture of TiI_2 and TiI_3 can be recovered from the reaction vessel. The proportions in the mixture depend on the temperature, since TiI_3 is appreciably more volatile than TiI_2 . At bar temperatures above $1100^\circ C$, TiI_4 is decomposed to Ti and I_2 . No evidence of intermediates in the decomposition has been obtained. By replacing the titanium bar with a tungsten one, the etching and deposition reactions of Ti have been separated. Estimates of activation energies for these reactions have been made.

INTRODUCTION

Experimental details for the refining of crude chromium, copper, hafnium, titanium, vanadium, and zirconium have been worked out using the van Arkel technique (2, 3) of a hot wire with an iodide as a carrier gas. The dimensions of the apparatus, the optimum filament temperatures and the optimum container temperatures have been established experimentally. As yet, however, a clear concept of the factors, as they affect the deposition of titanium metal and the appearance of slightly volatile sub-iodides of titanium in the vessel, has not been offered. The experimental work reported here was done in an attempt to elucidate some of these factors through a study in a flow system. In such a system, the reaction products from a series of runs at different temperatures and pressures can be removed and determined readily.

EXPERIMENTAL

The apparatus shown diagrammatically in Fig. 1 was constructed of pyrex and, through two conventional liquid air traps, was fitted to a pumping system capable of reducing the pressure in the system rapidly to 10^{-5} mm. of mercury.

A weighed sample of 99.5% TiI_4 (produced by the reaction of I_2 with an excess of hot Ti powder) was stored in a container (D) with a thin wall at (C). After the apparatus had been degassed, and the reaction vessel, connecting tubing, and TiI_4 bulb had reached the desired temperature, a magnetically operated iron-in-pyrex breaker (A) was dropped, and the reaction vessel was flooded with TiI_4 vapor. A capillary outlet (E) to the traps reduced the outgoing flow of TiI_4 and products, so that the pressure in the reaction vessel was controlled by the temperature of the TiI_4 source, and, in fact, approached the equilibrium vapor pressure of TiI_4 at the source temperature. It will be noted that the corrosive properties of TiI_4 do not permit the use of conventional manometers.

The TiI_4 vapors enter the reaction vessel at (K) and pass vertically over an

¹ Manuscript received June 10, 1952.

Contribution from the Department of Metallurgical Engineering, University of Toronto, Toronto, Ont. Published with permission of the Defence Research Board of Canada.

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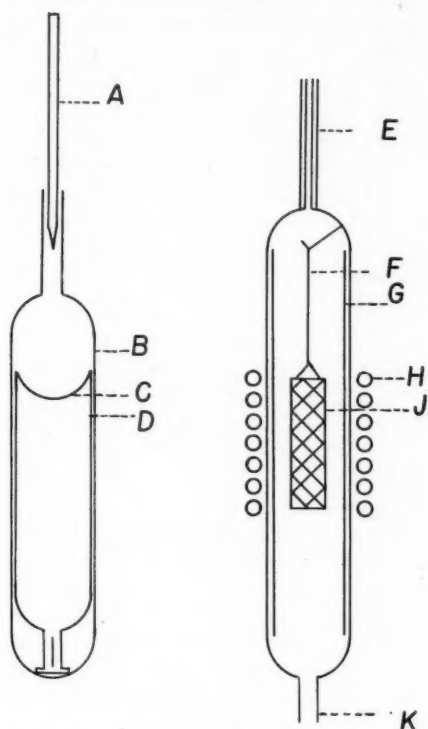


FIG. 1.

induction heated bar (J), either of 98% pure Ti or of pure tungsten. The bars were of the same surface area. The bar is supported in the field of an induction coil (H) by a molybdenum suspension (F). Excessive loss of heat and collapse of the pyrex vessel at high temperatures in the region of the bar are prevented by a transparent silica liner (G). The bar is removed for weighing through the bottom of the apparatus near (K). The same apparatus was used throughout a series of experiments.

RESULTS AND DISCUSSION

Experiments Using a Titanium Bar

When the TiI_4 source is held at a constant temperature of 205°C ., the equilibrium TiI_4 pressure is about 20 mm. (1). At Ti bar temperatures from 800°C . to 1000°C ., this pressure of TiI_4 causes a marked etching of the Ti bar. The rate of weight decrease in $\text{mgm./cm}^2 \cdot \text{hr.}$ is shown in Fig. 2. The reaction product, TiI_2 , collects on the walls of the silica liner of the reaction vessel. The TiI_2 is not pure, however, since a further side reaction with TiI_4 yields TiI_3 .



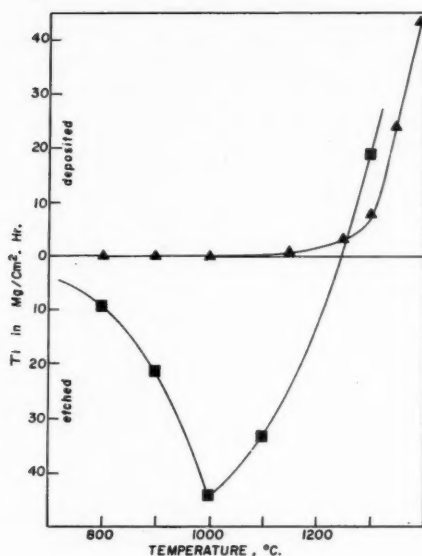


FIG. 2.

The composition of the residue in the reaction vessel varied with the temperature of the Ti bar. As the temperature of the bar (and the walls of the reaction vessel) was increased, conditions were more favorable for the evaporation of TiI_3 and less favorable for its formation. Thus, at 1100°C ., the material recovered, having an iodine:titanium ratio of 2.01:1.00, was TiI_2 only.

At low Ti bar temperatures, the material recovered in the trap consisted mainly of TiI_4 , as shown by an iodine:titanium ratio of 3.72:1.00 at 800°C . With increasing bar temperature, and the resulting increased transfer of TiI_3 , the ratio decreased to 3.25:1.00 at 1000°C . No free iodine was recovered within this temperature range.

An activation energy of 19.8 ± 0.5 kcal. has been estimated over the temperature range 800 - 1000°C . for reaction 1 by considering the rate of Ti removal from the bar as it varied with increasing bar temperature.

Additional experiments done at 1000°C . with increased TiI_4 pressures have shown that, when the pressure is doubled to 40 mm., the rate of Ti etching is increased by only 25%. A further increase to 80 mm. alters the rate by an additional 5%. This tendency for the reaction rate to become independent of pressure at higher pressures confirms the heterogeneity of the etching reaction and suggests that the optimum economic rate of sub-iodide formation would be obtained near pressures of 20 mm. TiI_4 vapor.

When the temperature of the Ti bar was increased above 1000°C ., there was evidence of nonuniformity of temperature over the surface of the bar. Near the edges of the bar, the etching of titanium continued, though at reduced rate.

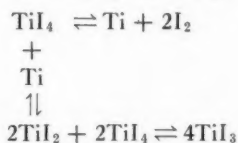
Toward the center of a bar face, the deposition of new titanium metal was evident. As the temperature was further increased, the rate of etching and the recovery of TiI_4 were decreased, while the rate of Ti deposition was increased. From Fig. 2, it is apparent that, at a temperature of $1250^\circ C.$, the bar was in equilibrium, relative to weight gain or loss, with its surroundings. Above $1250^\circ C.$, there was no evidence of etching, and the deposit of Ti, although it covered the bar completely, was heavier on the bottom. At bar temperatures of $1250^\circ C.$, there was no evidence of sub-iodide formation in the reaction vessel. Contents of the trap showed an iodine to titanium ratio of 4.79:1.00, thus indicating the liberation of free iodine as Ti was deposited. Iodine liberation was in quantitative agreement with Ti deposition.

Experiments Using a Tungsten Bar

To separate the etching and deposition reactions of titanium by not having titanium present at temperatures where it might be etched away, the titanium bar was replaced by a tungsten bar of the same surface area. Experiments were essentially duplicates of those done at the same temperatures with the titanium bar. The results, plotted in Fig. 2, show that appreciable deposition of Ti does not begin until a bar temperature of $1100^\circ C.$ has been reached. The rate of Ti deposition increased rapidly with temperature. The deposit was uniform, finely crystalline, and bright. There was no indication of sub-iodide formation, and since the only apparent products are iodine and titanium, it must be assumed that if the decomposition of TiI_4 proceeds stepwise through sub-iodide formation, then the flow rates used in these experiments were not sufficiently high to remove the intermediate products from the reaction zone before complete degradation to titanium and iodine had taken place. An activation energy of 92.0 ± 2 kcal. has been estimated for the deposition of Ti on a tungsten bar.

SUMMARY

Titanium tetraiodide in the presence of titanium, as used in these experiments, may be represented in the following series of equilibria:



At Ti bar temperatures below $1100^\circ C.$, all equilibria are displaced counterclockwise; above $1100^\circ C.$ the clockwise displacement results in Ti disposition and the liberation of free I_2 .

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THE CONSTITUTION OF A POLYURONIDE HEMICELLULOSE FROM WHEAT STRAW¹

By G. A. ADAMS²

ABSTRACT

The structure of a polyuronide hemicellulose from wheat straw containing xylose, arabinose, and hexuronic acid units has been investigated. Graded hydrolysis preferentially removed anhydro-L-arabinose units leaving a xylan to which uronic acid units were attached. Methylation and hydrolysis yielded 2,3,4-trimethyl xylose (2.7%) indicating one nonreducing end group per 37 units; 2,3,5-trimethyl-L-arabinose, 2,3-dimethyl-D-xylose, and 2-methyl-D-xylose were found in a molar ratio 1:5:1. In addition, a methylated uronic acid complex was recovered which on extensive hydrolysis yielded an aldobiuronic acid. The latter, on reduction with sodium borohydride, yielded 2,3,4-trimethyl glucose (1 mole) and 2-methyl xylose (1 mole); the structure 2-methyl 3[2,3,4-trimethyl α -D-glucuronosido] D-xylose was therefore indicated for the methylated aldobiuronic acid.

A structure for the hemicellulose is proposed which consists of approximately 32 anhydro-D-xylose units linked β 1,4- in a chain to which five anhydro-L-arabinose and three D-glucuronic acid units are attached as side groups by 1,3-glycosidic bonds. Results of periodate oxidation and estimation of reducing power support the proposed structure.

Although xylans have been found in practically all land plants, no certainty exists that they are composed entirely of anhydro-D-xylose units; L-arabinose units as well as D-glucuronic acid units may also be present as minor constituents of the molecule (3, 10). In a recent review, Whistler has pointed out that the existence of more than one type of xylan in nature is uncertain, although two types, one with and one without D-glucuronic acid, are indicated (17). A study of the constitution of a hemicellulose of New Zealand flax showed that the uronic acid component formed the nonreducing end group of a xylan chain (9). A similar position is occupied by D-glucuronic acid in the xylan of pear cell wall (8). Crude hemicelluloses of alfalfa hay (14), wheat straw (15), and corn stalks (16) have been shown to contain D-xylose, L-arabinose, and D-glucuronic acid. The molar ratio of the three components in hemicellulose B of wheat straw was 23:0.9:1. Chanda and his co-workers (7) established that esparto xylan consists of a singly branched molecule containing approximately 75 D-xylopyranose units united by 1,4-glycoside bonds with the branch point formed by a 1,3 linkage. Rigorous purification of the xylan removed the arabinose units which were thought previously to be attached as end groups.

The isolation from wheat straw of a hemicellulose containing a constant proportion of D-xylose, L-arabinose, and a hexuronic acid after purification (1, 2) led to the present investigation of the constitution of this relatively complex polysaccharide.

Studies (6, 11) on polysaccharide materials composed of D-xylose and L-arabinose have shown that mild methods of hydrolysis preferentially removed

¹ Manuscript received May 20, 1952.

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 141 on the Uses of Plant Products and as N.R.C. No. 2805.

² With the technical assistance of A. E. Castagne.

most of the L-arabinose units without appreciably affecting the xylan chain. In the present investigation, graded hydrolysis with 0.05 *N* oxalic acid released over 90% of the arabinose units from the polysaccharide in two hours. The acid lability of the arabinose indicated that it was in the furanoside configuration while the stability of the xylan suggested the usual pyranoside form. The ready removal of arabinose units without concomitant release of xylose tentatively suggested the existence of a xylan chain to which arabinose units were attached as side chains. The original polysaccharide was soluble in the dilute oxalic acid solution but progressive removal of arabinose side chains caused precipitation of a small fraction which consisted only of xylose residues. This observation parallels that made on wheat flour pentosans by Perlin (11). A further portion of the material, precipitable by ethanol, also contained only xylose units. Direct attachment of the uronic acid to the main xylan chain was demonstrated by the recovery of xylans with their original uronic acid content.

Complete methylation of the hemicellulose and fractionation with chloroform-petroleum ether yielded a product having $[\alpha]_D^{20} - 88.6^\circ$ (*c*, 0.5 in chloroform) and OCH_3 , 39.0% (calculated for $\text{C}_7\text{H}_{12}\text{O}_4 \cdot \text{OCH}_3$, 38.8%). Hydrolysis of the methylated hemicellulose formed the following products which were estimated and identified as described in the experimental section: (I) 2-methyl-D-xylose; (II) 2,3-dimethyl-D-xylose; (III) 2,3,4-trimethyl-D-xylose; (IV) 2,3,5-trimethyl-L-arabinose; (V) 2-methyl 3-[2,3,4-trimethyl-D-glucuronosido] D-xylose.

As a result of the methylation studies, certain features of the molecule become evident. The identification of 2,3,5-trimethyl methyl-L-arabinoside proved that it was an end group joined to the other units in the polysaccharide by glycosidic bonds. The ready cleavage of the arabinose units by mild acid hydrolysis was evidence of the furanose form of the sugar. The yield of trimethyl arabinose was approximately equal to the arabinose content of the original hemicellulose and hence this pentose was attached wholly in end-groups positions. The presence of arabinose in the form of an araban seems unlikely, since mono- and dimethyl arabinosides were not found among the methanolysis products.

The methylated aldobiuronic acid, after reduction with borohydride and subsequent hydrolysis, was shown to yield equimolar quantities of 2-methyl xylose and 2,3,4-trimethyl glucose. These observations show that $\text{C}_{(3)}$ of the xylose was involved in the union with 2,3,4-trimethyl glucuronic acid, and that the methylated aldobiuronic acid was therefore 2-methyl 3-[2,3,4-trimethyl-D-glucuronosido] D-xylose. The positive rotation of the methyl ester of the aldobiuronic acid glycoside ($[\alpha]_D^{20} + 73.3^\circ$) suggested an α glycosidic link between the uronic acid and D-xylose. The identification of 2,3,4-trimethyl glucose as a reduction product showed that the uronic acid occupied the position of a non-reducing end group and was linked glycosidically to a xylose unit. The assumption is made that this xylose unit was linked to the others in the xylan chain by the usual β 1,4-linkage. No specific position in the chain can be assigned to the D-glucuronic side groups but it is clear that they cannot be attached to the non-reducing end groups.

The nonreducing end group of the xylan chain appeared in the methanolysis

mixture as 2,3,4-trimethyl-D-xylose. The amount of this substance (2.7%) indicated approximately 37 xylose units per nonreducing end group.

Recovery of a high proportion of the methylated sugars as 2,3-dimethyl-D-xylose proved that the xylose units were linked 1,4 and were in the pyranose form. It was also evident that only β linked xylose residues were involved as the specific rotation of the methylated polysaccharide was highly negative (-88°) and changed to positive ($+58^\circ$) on hydrolysis. This general structure of xylan chains has been established for esparto xylan (7), pear cell wall xylan (8), and hemicellulose of New Zealand flax (9).

Methylated sugars I, II, III, and IV occurred in a molar ratio of 5:25:1:5. The amount of glucuronic acid in the hemicellulose molecule, as calculated from the uronic acid content of the original polysaccharide, was three moles. From the foregoing data the hemicellulose molecule was envisaged as a chain structure of approximately 29 D-xylopyranose units joined by β 1,4-linkages and terminated by one reducing and one nonreducing group. To this straight chain five L-arabinose and three D-glucuronic acid units were attached by 1,3-glycosidic bonds. The attachment of the side groups to C₍₃₎ of the xylan chain was deduced from the fact that 2-methyl-D-xylose was the only monomethyl xylose isolated from the methanolysis products. The number of moles of 2-methyl-D-xylose, indicating the number of branch points, equalled the number of moles of 2,3,5-trimethyl arabinose which were side groups. The attachment of three glucuronic acid units to the main xylan chain should yield a corresponding number of monomethyl xylose residues but these did not appear after methanolysis and hydrolysis because they were attached to the uronic acid as highly resistant aldobiuronic acid units. These three residues provided a total of 32 xylan residues which with five arabinose and three glucuronic acid residues gave a total of 40 residues in the molecule.

Estimation of reducing power by the Somogyi copper reduction method indicated a molecule with one reducing group in 32-34 units. Although this result is somewhat lower than that required by the proposed structure, a molecule of the same general order of magnitude is indicated. Hypiodite oxidation of the hemicellulose yielded results which suggested a molecule of only 10-12 units. This reaction is difficult to control and leads to overoxidation; hence the results are not reliable (7).

Further evidence of the proposed structure for the hemicellulose was obtained by periodate oxidation which showed that the polysaccharide consumed approximately 0.83 moles of periodate per mole ($C_5H_8O_4$). The proposed structure, theoretically, should consume 0.87 moles of periodate per mole. The amount of formic acid produced was approximately 0.08 moles per anhydropentose residue, corresponding to 1 mole per 12 sugar residues. The sources of formic acid were, 1 mole from the nonreducing xylose end group and two from the xylose reducing end group. Thus, on the basis of formic acid production the molecule should contain 36 units. Formic acid production from periodate oxidation of the proposed molecule should be 0.077 moles per mole.

Earlier investigations (1) indicated that the uronic acid component of the

hemicellulose contained one methoxyl group. Periodate oxidation results supported this observation. Unmethylated uronic acid would have consumed 2 moles of periodate per mole and produced 1 mole of formic acid per mole; the data presented indicate that the uronic acid units consumed only 1 mole of periodate and produced no formic acid. Because of the periodate consumption, it may be inferred that the methoxyl is not on $C_{(3)}$. Since $C_{(4)}$ is involved in lactone formation, $C_{(2)}$ appears to be the point of attachment.

Although the occurrence of a hemicellulose fraction of wheat straw composed of D-xylose, L-arabinose, and D-glucuronic acid has been reported previously (15), the present investigation appears to be the first attempt to assign a structure to the molecule. Considerable difficulty has arisen in deciding whether wood and straw xylans actually contain L-arabinose. Extensive purification of esparto xylan (7) has demonstrated that the arabinose hitherto associated with xylan by earlier workers (6) was an extraneous impurity which could be removed. On the other hand, Boggs *et al.* (5) recently reported that wheat straw xylan contained arabinose in nonterminal positions. The structure of a polyuronide hemicellulose from New Zealand flax has been shown to consist of a main xylan chain of 9-10 units to which glucuronic acid was attached as a nonreducing group (9). The constitution of pear cell wall xylan is somewhat similar although the molecule is larger and singly branched (8).

Evidence seems to be accumulating that "xylans" from various plants are not identical and that even within one species more than one type may exist. Successive extractions of hemicellulose from corn stalks with different solvents has yielded products with varying pentosan:uronic acid ratios and different apparent molecular weights (4). A similar investigation of wheat straw holocellulose has yielded a series of hemicelluloses which vary in xylose, arabinose, and uronic acid content (1). Clearly, more studies are needed to establish the fine structure of the various "xylans" as well as their distribution in plants.

EXPERIMENTAL

Preparation of the hemicellulose used in the following investigations has been described in detail in previous publications (1, 2). Component sugars obtained by acid hydrolysis were separated by paper chromatography (1) and determined by the micro-method of Somogyi (12). Uronic acid was estimated by the micro-method of Tracy (13). Nitrogen content was determined by the micro-Kjeldahl procedure.

The composition of the hemicellulose was as follows: nitrogen, 0.21%; uronic acid anhydride, 10.6%; D-xylose, 71.7%; L-arabinose, 13.6%; $[\alpha]_D^{25} - 92^\circ$ (c, 1.0% in 2.5% sodium hydroxide).

Graded Hydrolysis

Three conditions of hydrolysis were investigated: autohydrolysis at 96°C .; 0.05 *N* sulphuric acid at 96°C .; 0.05 *N* oxalic acid at 96°C . Heating of the hemicellulose (50 mgm.) in water (5 ml.) at 96°C . and chromatographic investigation of the hydrolyzate at the end of hourly heating periods showed that no hydrolysis had occurred within six hours. This method of graded hydrolysis was discontinued as ineffective.

Hydrolysis with 0.05 *N* sulphuric acid at 96°C. proved too drastic as considerable quantities of xylose were released along with the arabinose within a 30 min. heating period.

Preliminary experiments with 0.05 *N* oxalic acid at 96°C. showed that only arabinose was released from the polysaccharide in one and two hour heating periods. In further experiments, the polysaccharide was hydrolyzed with 0.05 *N* oxalic acid for 2, 3, 4, 5, 8, and 12 hr. to determine the heating period required for maximum amount of arabinose obtainable with minimum removal of xylose. The results given in Table I indicate that about 92% of the total arabinose and

TABLE I
GRADED HYDROLYSIS OF HEMICELLULOSE WITH 0.05 *N* OXALIC ACID
(Sugar content: xylose 44.4 mgm.; arabinose, 7.78 mgm.)

Sugars in hydrolyzate	Time of hydrolysis, hr.					
	2	3	4	5	8	12
Arabinose, mgm.	7.26	7.50	7.78	7.78	7.82	7.78
Xylose, mgm.	1.56	2.46	4.11	5.44	10.9	15.5

3.5% of the total xylose were released in a two hour heating period. A more detailed examination of the hydrolytic products was made possible by the following experiment: hemicellulose (0.40 gm.) was hydrolyzed in oxalic acid (100 ml. 0.05 *N*) for two hours at 96°C. The solution was centrifuged and the residue recovered. Addition of ethanol to the solution precipitated a white flocculent product which was thoroughly washed with water. Hydrolysis of the residue and precipitated material showed that the sugars in the hydrolyzates were 97 and 98% xylose respectively. In addition, the uronic acid anhydride content of the residue and the alcoholic precipitate were found to be 11.0 and 10.8% respectively.

Methylation

Hemicellulose (21.9 gm.) was placed in a four necked flask fitted with two dropping funnels, a mechanical stirrer, and a gas inlet. The hemicellulose was dissolved in water (100 ml.) and the air displaced by a slow stream of nitrogen. Sodium hydroxide (300 ml., 40%) and dimethyl sulphate (150 ml.) were then added simultaneously over a period of four hours with vigorous stirring. Conditions were kept mildly alkaline (pH 8.0 – 8.5) until the end of this period when the reaction was allowed to become strongly alkaline (pH 7 – 13.0); stirring was continued for a further period of 12 hr. The reaction mixture was then placed in an ice bath and the pH of the solution reduced to approximately 7.0 with sulphuric acid (50%). The partially methylated polysaccharide and inorganic salts which settled out were centrifuged off. The supernatant solution was concentrated under reduced pressure to a thick paste and recombined with the centrifuged solids. The methylation procedure was repeated twice in the same way except for the addition of acetone (75 ml.) to dissolve the partially methylated polysaccharide. After the third methylation the mixture was neutralized as before and then poured into 5 liters of warm water and dialyzed in cellophane bags for 40 hr. against tap water to remove the excess of inorganic salts. The

dialyzed solution was concentrated at 50°C. under reduced pressure to a brown syrup (OCH_3 , 28.3%). Three additional methylations were carried out (six methylations in all) and the partially methylated product recovered again as a brown gum after dialysis (OCH_3 , 33.0%). This material was now soluble in acetone, partially soluble in chloroform, and more soluble in cold water than in hot water. Three further methylations were carried out and the methylated polysaccharide recovered as a brown solid (OCH_3 , 36.8%), readily soluble in methyl iodide-methanol mixture (4:1) and partially soluble in chloroform.

The methylated product (21.3 gm.) was placed in a three-necked flask and dissolved in methyl iodide (200 ml.) and methanol (50 ml.). Over a period of four hours, silver oxide (80 gm.) was added while the mixture was stirred and heated under reflux at 45°C. After standing overnight the silver salts were removed by centrifuging and the solids extracted with chloroform. The solution and chloroform washings were concentrated to a yellow resin. After two further methylations, the product was isolated after drying as a yellowish powder, yield 18.5 gm.; OCH_3 , 39.0%; (theoretical value for dimethyl xylan 38.8%); $[\alpha]_D^{25} - 87.2^\circ$ (c, 0.5 in chloroform). One additional methylation treatment caused no further increase in methoxyl content.

Fractionation of the Methylated Polysaccharide

The usual method of fractionating methylated polysaccharides from chloroform solution with petroleum ether was unsatisfactory because of the large volumes of petroleum ether involved and the difficulty of recovering small fractions. It was found more advantageous to selectively extract fractions from the methylated product with various mixtures of chloroform and petroleum ether. The methylated polysaccharide (18.5 gm.) was extracted under gentle reflux with the solvent mixture and decanted through a glass filter ("C" porosity). The solvent was removed under reduced pressure and the product dried *in vacuo* at 50°C. The results of the fractionation are given in Table II. Approximately 85% of the product was in fraction 4 and was fully methylated; this material was used in subsequent studies.

TABLE II
FRACTIONATION OF METHYLATED HEMICELLULOSE BY
CHLOROFORM-PETROLEUM ETHER SOLUTION

Fraction No.	Solvent mixture chloroform-petroleum ether	Yield, %	Methoxyl content, %	$[\alpha]_D^{25}$
1	0 : 100	0.9	24.0	—
2	10 : 90	2.8	27.9	—
3	20 : 80	10.8	38.7	- 86.0°
4	30 : 70	84.6	39.1	- 88.6°

Hydrolysis Products of Methylated Hemicellulose

Fraction 4 (100 mgm.) was sealed in a glass tube with methanolic hydrogen chloride (5 ml.; 5%) and heated in a boiling water bath for 12 hr. After removal of the solvent, the methyl glycosides were recovered as a viscous brown syrup

which was hydrolyzed with hydrochloric acid (5 ml.; 0.5 *N*) by heating at 100°C. for eight hours. The acid solution was neutralized with silver carbonate, filtered, and the excess silver precipitated with hydrogen sulphide. After concentrating the solution to a thin syrup at 40°C., the sulphide precipitate was centrifuged off. The syrup was examined on a filter paper chromatogram using the solvent system *N*-butanol-ethanol-water-ammonia (40:10:49:1). Authentic samples of 2-methyl xylose, 2,3-dimethyl xylose, and 2,3,5-trimethyl arabinose were used as reference compounds. Development of the chromatograms with aniline phthalate showed the presence of (1) 2,3,5-trimethyl arabinose (R_f 0.81 – 0.83); (2) 2,3-dimethyl xylose, (R_f 0.66 – 0.69); (3) 2-monomethyl xylose (R_f 0.43 – 0.47); (4) two adjacent pink spots (R_f 0.09 – 0.12; 0.15 – 0.17) indicating the presence of uronic acids.

Quantitative Estimation of Methylated Sugars

The foregoing qualitative experiment was repeated on a quantitative basis. The sugars extracted from the chromatograms were estimated by alkaline hypiodite oxidation (7). The results are given in Table III.

TABLE III
COMPOSITION OF HYDROLYZATE FROM METHYLATED HEMICELLULOSE

Component	Molar composition, %
Monomethyl pentose	12.5
Dimethyl pentose	62.3
Trimethyl pentose	15.1

Separation of Methanolysis Products

Since the foregoing experiments tentatively identified the methylated sugars and estimated the amounts present in the methylated polysaccharide, the following experiments were carried out to provide sufficient quantities of the individual sugars for characterization. Fraction 4 (fully methylated polysaccharide, 9.5 gm.) was hydrolyzed with methanolic hydrogen chloride (350 ml.; 5%) to constant rotation (58°). The methanolysis mixture was adjusted to pH 7.0 with silver carbonate, filtered, and the precipitate washed with hot methanol. The filtrate and washings were concentrated to yield a brown syrup (9.3 gm.), taken up in anhydrous ether, and the small residue of inorganic and tarry substances removed by centrifuging. The final yield of syrup was 9.2 gm. Saturated barium hydroxide (100 ml.) was added to the syrup and the mixture heated for three hours at 60°C. on a steam bath. Excess barium hydroxide was removed by carbon dioxide, the solution heated for 15 min. at 85°C. to decompose any barium hydrogen carbonate, filtered, and the precipitate thoroughly washed with hot water. The aqueous solution was concentrated to about 150 ml. under reduced pressure.

Preliminary experiments with small batches of the methanolysis products showed that the fully methylated glycosides (trimethyl pentosides) were soluble in *n*-pentane. The dimethyl pentoside was preferentially soluble in

ethyl ether and the monomethyl pentoside was extractable by chloroform. Since the barium salt of the uronoside was insoluble in the foregoing solvents, it remained in aqueous solution.

The aqueous mixtures of glycosides and uronoside were successively extracted with *n*-pentane, ethyl ether, and chloroform in continuous liquid-liquid extractors. Fig. 1 is a diagram of the separation scheme. Periodically during the

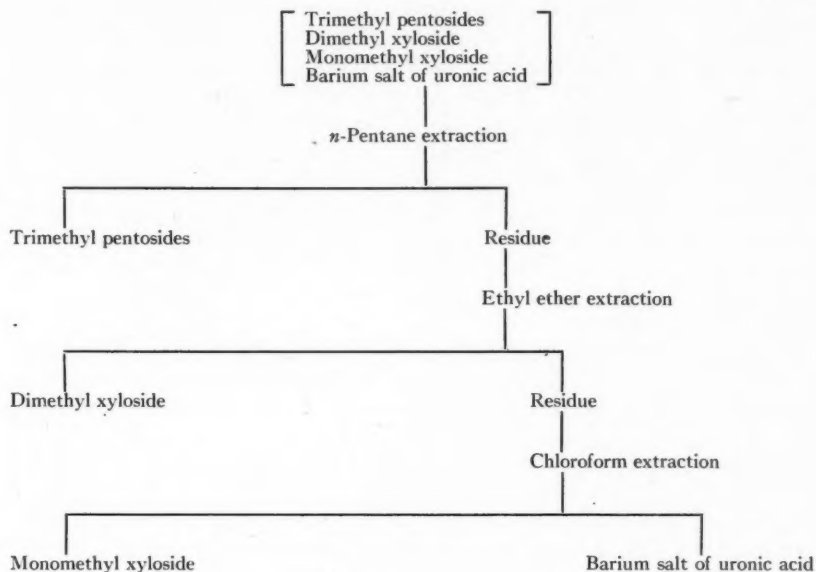


FIG. 1. Separation of methanolysis products.

extractions, small samples of the extracted glycosides were hydrolyzed and the free sugars identified by paper chromatography. By this means of control, relatively pure methyl glycosides were obtained. After extraction of the glycosides, the aqueous solution was evaporated to dryness under reduced pressure. The yield, methoxyl content, and specific rotation of the fractions are given in Table IV.

TABLE IV
YIELD AND PROPERTIES OF PENTOSIDES AND URONOSIDE FRACTION

Fraction	Product	Wt., gm.	OCH ₃ , %	$[\alpha]_D^{20}$ (Chloroform)
1	Trimethyl pentoside	1.14	58.8	- 58.0°
2	Dimethyl pentoside	4.86	47.6	+ 41.5°
3	Monomethyl pentoside	1.04	33.1	+ 78.0°
4	Barium salt of methylated uronoside	1.48	38.6	—

*Examination of the Various Methanolysis Fractions**Fraction 1*

Hydrolysis of fraction 1 (trimethyl methylpentoside; 50 mgm.) with hydrochloric acid (0.5 *N*) yielded a solution having a final $[\alpha]_D^{25} + 4.5^\circ$. This result indicated that the product was not pure 2,3,5-trimethyl arabinose. Chromatography of the free methylated sugars in butanol-ethanol-water showed two spots which were very closely associated. Structural considerations suggested the presence in the solution of both 2,3,4-trimethyl xylose and 2,3,5-trimethyl arabinose.

Estimation and Separation of Trimethyl Xylose and Trimethyl Arabinose

Fraction 1 (49 mgm.) was hydrolyzed at 100°C. with hydrobromic acid (5 ml.; *N*/25) until the optical rotation became constant (six hours). This procedure preferentially hydrolyzed the arabinoside with little effect on the xyloside. Addition of concentrated hydrochloric acid (0.2 ml.) and a further heating period of six hours hydrolyzed the xyloside component. Analysis of the reducing power at the end of the partial and the complete hydrolysis periods showed that 84% of the total sugar was trimethyl arabinose and 18% trimethyl xylose. The composition of the trimethyl pentose fraction was therefore trimethyl xylose 2.7% and trimethyl arabinose 12.4%.

Separation of the trimethyl sugars was accomplished using graded hydrolysis in the following way: Fraction 1 (714 mgm.) was hydrolyzed at 100°C. in hydrobromic acid (25 ml.; *N*/25) to constant rotation. The hydrolyzate was brought to pH 6.0 with sodium hydroxide and extracted with *n*-pentane in a liquid-liquid extractor. This procedure removed the trimethyl methylxyloside (Fraction 1a; 88 mgm.) from the free methylated arabinose. Chromatographic examination of this fraction after hydrolysis showed a small amount of dimethyl xylose also present. Evaporation of the aqueous residue yielded the trimethyl arabinose (Fraction 1b).

Fraction 1a

Further extractions of this syrup from aqueous solution with *n*-pentane reduced the dimethyl xylose content and yielded trimethyl methylxyloside ($[\alpha]_D^{20} - 80^\circ$) (61 mgm.). Hydrolysis of the syrup with hydrochloric acid (10 ml. 0.5 *N*) was carried out by heating at 100°C. for six hours. Neutralization with silver carbonate in the usual way yielded a yellowish syrup (48 mgm.) which was decolorized with charcoal. Paper chromatography showed a slight trace of dimethyl xylose in the main trimethyl xylose fraction. The syrup was taken up in ether and a few drops of petroleum ether added; on seeding with 2,3,4-trimethyl-D-xylopyranose, crystallization occurred. After isolation and recrystallization from methanol, 2,3,4-trimethyl-D-xylopyranose was recovered, m.p. 89-90°C. (undepressed on admixture with an authentic sample), and $[\alpha]_D^{20} + 20.0^\circ$ (*c*, 0.7 in water).

Analysis: Calculated for $C_8H_{16}O_5 \cdot OCH_3$, 48.4.

Found: OCH_3 , 48.2.

Fraction 1b

On chromatographic examination this fraction showed only one spot which

corresponded to an authentic sample of 2,3,5-trimethyl-L-arabinose. The syrup (391 mgm.) was taken up in acetone and some brownish residue filtered off. Treatment in chloroform solution with charcoal yielded a syrup (305 mgm.) having an η_D^{20} 1.4522; $[\alpha]_D^{20} - 36^\circ$; OCH_3 47.6% (calculated for $\text{C}_8\text{H}_{16}\text{O}_5$, 48.4%). These data identify the syrup as 2,3,5-trimethyl-L-arabinose. The amide of this sugar was prepared as follows: the methylated sugar (183 mgm.) was dissolved in water (1 ml.), bromide (eight drops) added, and the mixture then heated for one hour at 50°C . and let stand overnight at 20°C . After removal of the excess bromine by aeration, the solution was neutralized with silver carbonate and filtered; excess silver was removed with hydrogen sulphide. The solution was evaporated to dryness and 2,3,5-trimethyl arabonolactone recovered as a syrup. Treatment with methyl alcoholic ammonia at room temperature converted the lactone into the amide of 2,3,5-trimethyl-L-arabonic acid which crystallized on nucleation. Recrystallization from methanol-ether solution yielded the pure amide, m.p. 138°C . (undepressed with an authentic sample) and $[\alpha]_D^{25} - 17.9^\circ$ (c , 1.64 in water).

Analysis: Calculated for $\text{C}_8\text{H}_{17}\text{O}_5\text{N}$: OCH_3 , 44.9.

Found: OCH_3 , 44.4.

Fraction 2

A portion of the syrup (987 mgm.) was dissolved in hydrochloric acid (25 ml., 0.5 *N*) and heated at 100°C . The specific rotation changed from $+41.5^\circ$ (initial value) to $+26.3^\circ$ (final value) in four hours. The solution was neutralized with silver carbonate and the free sugar recovered in the usual way. The syrup was taken up in ether, filtered, and reconcentrated. Yield 877 gm.; $[\alpha]_D^{20} + 24^\circ$ (c , 1% in water); η_D^{20} 1.4733.

Analysis: Calculated for $\text{C}_7\text{H}_{14}\text{O}_5$: OCH_3 , 34.8.

Found: OCH_3 , 34.8.

The syrup (282 mgm.) was taken up in methanol (5 ml.), freshly distilled aniline (1 ml.) was added, and the mixture refluxed for 2.5 hr. After removing the solvent and allowing the reaction mixture to stand about a week, the anilide was recovered in crystalline form. On recrystallization from ethyl acetate, the anilide of 2,3-dimethyl-D-xylose was obtained. The melting point ($123\text{--}124^\circ\text{C}$.) was undepressed when admixed with the authentic anilide. Examination of the crystals by the X-ray powder photograph method showed them to be the anilide of 2,3-dimethyl-D-xylose.

Fraction 3

The free sugar extracted from the methanolysis mixture by chloroform on purification yielded a syrup (OCH_3 , 34.5) (calculated for $\text{C}_7\text{H}_{14}\text{O}_5$: OCH_3 , 34.8). On hydrolysis with hydrochloric acid (0.5 *N*) the specific rotation changed from 78° (initial value) to $+32^\circ$ (final value) in three hours. Neutralization of the acid with silver carbonate and recovery of the product yielded a brown syrup. After treatment with charcoal, the syrup was almost white and partially crystallized after seeding with 2-monomethyl xylose. Recrystallization from methanol-ethyl ether solution yielded 2-monomethyl-D-xylose, m.p. 133° ; $[\alpha]_D^{20} + 34^\circ$; (c , 1% in water).

Analysis: Calculated for $C_6H_{12}O_5 \cdot OCH_3$, 18.9%.

Found: OCH_3 , 18.6%.

Conversion to the anilide gave a crystalline product, m.p. 123°, alone or admixed with an authentic sample of 2-methyl-D-xylose anilide.

Fraction 4

The barium salts separated from alkaline hydrolysis of the methanolysis syrup were refluxed several times with ether to remove residual pentoside fractions. The ether insoluble residue (500 mgm.) was sealed in a glass tube with methanolic hydrogen chloride (15 ml.; 5%) and heated in a boiling water bath for seven hours. After neutralization with silver carbonate, the filtered solution, on removal of the solvent, yielded a yellow syrup (324 mgm.) OCH_3 ; 43.8%; $[\alpha]_D^{20} + 85^\circ$ (c, 1.0 in ethanol). A sample of this syrup (10 mgm.) was hydrolyzed with 0.5 N hydrochloric acid and the free sugars were chromatographed in a butanol-ethanol-water solvent system. Three spots were identified with aniline phthalate, namely: methylated uronic acid, 2-monomethyl xylose, and 2,3-dimethyl xylose.

To ensure complete methylation the product was subjected to two methylation treatments using Purdie's reagent (methyl iodide and silver oxide). The product was recovered as a brown gum (OCH_3 , 46.2%) and subjected to methanolysis treatment in methanolic hydrogen chloride (5.7%) under reflux for 43 hr. The methanolysis mixture, after the usual neutralization treatment, was heated with saturated barium hydroxide solution (20 ml.) for three hours at 60°C. to convert the uronic acid ester into the barium salt. After removal of excess barium hydroxide with carbon dioxide the aqueous solution was extracted for 24 hr. with ethyl ether in a continuous liquid-liquid extraction. Evaporation of the aqueous solution yielded the barium salt which was further extracted with ethyl ether under reflux. Chromatographic examination of the ether soluble fraction showed that some of the uronic acid material was soluble.

Chromatographic Separation of Methylated Uronic Acid Complex and Methyl Pentosides

Since solvent extraction did not lead to satisfactory separation of the barium salt of the uronic acid complex and the methyl pentosides, the following procedure was used. The ether extracts and the insoluble residue were first recombined and evaporated to dryness. The mixture was then taken up in hydrochloric acid (0.5 N) and heated at 100°C. for two hours. After neutralization with silver carbonate the solution was concentrated to a syrup which was taken up in methanol. The syrup was heavily spotted on filter paper and chromatographed overnight in *n*-butanol-ethanol-water solvent system. Methylated pentoses and uronic acid were located on the paper by reference spots developed with aniline phthalate. The portion of the sheet containing the uronic acid was cut out, ground into a fine pulp in a Wiley mill, and extracted with hot ethanol. Evaporation of the ethanol yielded a yellow syrup composed of free methylated aldo-biuronic acid. This product was converted to the methyl ester, methyl glycoside, by heating for eight hours in methanolic hydrogen chloride (5%). Recovery in the usual way yielded a syrup (264 mgm.) which was dissolved in ethanol (25 ml.).

Reduction With Sodium Borohydride

Sodium borohydride (50 mgm.) was dissolved in ethanol (5 ml.) and mixed

with the alcoholic solution of the syrup (5 ml.). No visible reaction occurred and the mixture was allowed to stand overnight. Dilute acetic acid was added to decompose the hydride, and the pH of the solution was reduced from 9.0 to 7.0. The solution was evaporated to dryness, extracted with hot ether and, after removal of the solvent, yielded a syrup (45 mgm.). Hydrolysis of the syrup (20 mgm.) with hydrochloric acid (5 ml.; 0.5 *N*) for six hours yielded the free sugars. Chromatographic examination of the sugars using butanol-ethanol-water showed four spots: unreduced uronic acid; 2-monomethyl xylose; 2,3-dimethyl xylose and 2,3,4-trimethyl-D-glucose; the last three compounds were identified by authentic reference samples on the same chromatogram. The origin of 2,3,4-trimethyl-D-glucose could only have been 2,3,4-trimethyl-D-glucuronic acid and hence the identity of the uronic acid in the hemicellulose as D-glucuronic was established.

Further Examination of Fraction 4

The methylated glucuronic acid complex (fraction 4) (430 mgm.) was heated in a sealed tube with methanolic hydrogen chloride (30 ml.; 5%) for 22 hr. After neutralization the methyl pentosides and uronoside were recovered as a syrup (381 mgm.). Alkaline hydrolysis with barium hydroxide converted the uronosidic ester to the barium salt. Methyl pentosides (and some of the barium salt) were removed by repeated extraction of the salt with boiling ethyl ether. The barium salt was converted to the methyl ester at room temperature in methanolic hydrogen chloride (20 ml.; 5.0%). After neutralization with silver carbonate, the methyl ester of the methylated uronoside was recovered as a heavy yellow syrup (259 mgm.) $[\alpha]_D^{20} + 73.3^\circ$; equivalent, 497 (calculated for $C_{18}H_{32}O_{11}$, equivalent, 424). Since the product was still an aldobiuronic acid, it was subjected to further hydrolytic treatments with methanolic hydrogen chloride (5%) for 17 hr. followed by hydrochloric acid 0.5 *N* for 17 hr. at 100°C. Chromatographic examination of the hydrolyzate showed the presence of 2-monomethyl xylose and methylated uronic acid.

A larger scale chromatographic separation permitted recovery of the uronic acid fraction by extraction of the paper with methanol. The free acid was converted to the methyl ester, methyl glycoside, by heating with methanolic hydrogen chloride and recovered as a yellowish syrup (90 mgm.). The syrup was dissolved in methyl alcoholic ammonia (10 ml.) and allowed to stand at room temperature for 24 hr. On removal of the solvent a syrup was recovered having $[\alpha]_D^{20} + 86.5^\circ$. It was clear that the product was not the amide of 2,3,4-trimethyl-D-glucuronic acid ($[\alpha]_D^{20} + 138^\circ$) but probably the amide of the methylated aldobiuronic acid.

Calculated for $C_{16}H_{29}O_{10}N$: OCH_3 , 39.2; N, 3.5.

Found: OCH_3 , 40.6; N, 3.6.

After about one month, fine feathery crystals formed around the edge of the syrup. It has not been possible to recover these crystals in pure form.

Periodate Oxidation

The hemicellulose (100 mgm.) was dissolved in periodic acid solution (4.6 gm. of periodic acid in 100 ml. water and neutralized with sodium hydroxide using methyl red indicator). All oxidations (11) were done in low-actinic glassware

flasks at 16°C. At various time intervals, analyses were carried out for periodate consumption and formic acid production (7). The following results were obtained:

	Time, hr.				
	48	72	120	144	168
Moles of periodate consumed per $C_5H_8O_4$	0.68	0.74	0.79	0.83	0.83
Moles of formic acid produced per $C_5H_8O_4$	—	0.044	0.081	0.081	0.081

After the periodate oxidation was complete, the remainder of the solution was treated with butylene glycol and then dialyzed in cellophane bags against distilled water for 24 hr. Concentration of the solution *in vacuo*, followed by addition of alcohol, yielded a fine flocculent precipitate. Hydrolysis of the precipitate followed by chromatographic analyses showed the presence of xylose only. The xylose originated from branch points in the molecule which were not oxidized by periodate.

Reducing Power of Hemicellulose

Determination of reducing power was carried out by hypiodite oxidation in sodium hydroxide – disodium hydrogen phosphate (pH 11.40) for six hours (7). The results indicated one reducing unit per 10-12 pentose residues.

Another experiment in which the reducing power was determined by the copper reduction method of Somogyi (12) gave a value of one reducing group per 32-34 pentose units.

ACKNOWLEDGMENTS

The authors are indebted to Prof. E. L. Hirst, Department of Chemistry, University of Edinburgh, for supplying samples of 2,3,4-trimethyl xylose, 2-methyl xylose, 2-monomethyl xylose anilide, and 2,3,5-trimethyl-L-arabonamide. Thanks are also tendered Dr. W. H. Barnes, National Research Laboratories, Ottawa, for preparing X-ray diffraction diagrams of the anilide of 2,3-dimethyl-D-xylose.

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THE SYNTHESIS OF PYRIDOCARBAZOLES¹

BY MARSHALL KULKA AND R. H. F. MANSKE

ABSTRACT

7H-Pyrido(2,3-*c*)- (IX, R = H) and 7H-pyrido(3,2-*c*)carbazole (V, R = H) have been synthesized by unambiguous routes and shown to be identical with the products resulting from the Fischer-indole ring closure followed by dehydrogenation of 6- and 7-quinolylhydrazones of cyclohexanone respectively. The cyclization of N-substituted-1,2,3,4-tetrahydro-6- and -7-quinolylhydrazones of cyclohexanone followed by hydrolysis and dehydrogenation resulted in the linear polycyclic systems, 6H-pyrido(3,2-*b*)- (VIII) and 10H-pyrido(2,3-*b*)carbazole (IV) respectively. The 12 possible pyridocarbazoles have now been prepared.

The structure of the Skraup reaction product of 1-phenyl-5-amino-1-benzotriazole (X) has been established.

The fusion of the carbazole nucleus (I) at the *a*, *b*, and *c* positions with the pyridine ring (II) at the 2,3- and 3,4-positions results in 12 isomeric pyridocarbazoles, six belonging to the quinoline and six to the isoquinoline series. The synthesis of the isoquinoline series, namely 11H-pyrido(3,4-*a*)-, 11H-pyrido(4,3-*a*)-, 10H-pyrido(3,4-*b*)-, 6H-pyrido(4,3-*b*)-, 7H-pyrido(3,4-*c*)-, and 7H-pyrido(4,3-*c*)carbazole, has already been reported (9, 10). Of the pyridocarbazoles belonging to the quinoline series, namely 10H-pyrido(2,3-*b*)- (IV), 7H-pyrido(3,2-*c*)- (V), 11H-pyrido(3,2-*a*)- (VI), 11H-pyrido(2,3-*a*)- (VII), 6H-pyrido(3,2-*b*)- (VIII), and 7H-pyrido(2,3-*c*)carbazole (IX), three (VI, VII, and IX) have also been recently synthesized (2,3,6) though the structure of IX was not rigorously established. The purpose of this investigation was to complete the synthesis of the pyridocarbazoles by unambiguous methods.

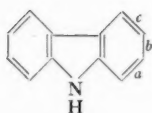
When 5-, 6-, 7-, and 8-quinolylhydrazones (III) of cyclohexanone undergo a Fischer-indole ring closure the products are tetrahydropyridocarbazoles which on dehydrogenation yield pyridocarbazoles. It will be noted that only 5- and 8-quinolylhydrazones can produce pyridocarbazoles of undoubted structure, the 6- and 7-quinolylhydrazones each being able to undergo cyclization in two directions. It was therefore necessary to block one of the two positions ortho to the hydrazono group in the latter cases before cyclization and to remove the blocking group afterwards, in order to accomplish unequivocal synthesis.

For this purpose 5,8-dichloro-6-quinolylhydrazones of cyclohexanone was prepared and subjected to the Fischer indole reaction. Ring closure was accomplished only under the most drastic conditions and the product was 5-chloro-8,9,10,11-tetrahydro-7H-pyrido(2,3-*c*)carbazole (tetrahydro-IX, R = Cl). Apparently the tendency of the 5,8-dichloro-6-quinolylhydrazones to ring close to the 5-position was so great that the chlorine in that position was eliminated to form the angular pyridocarbazole. The relative ease with which angular aromatic polycyclic compounds form as compared to the linear isomers is a well-known phenomenon (1). The dechlorination of 5-chloro-8,9,10,11-tetrahydro-7H-pyrido(2,3-*c*)carbazole (tetrahydro-IX, R = Cl) yielded tetrahydro-IX (R = H)

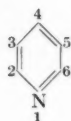
¹ Manuscript received May 8, 1952.

Contribution from the Dominion Rubber Company Limited Research Laboratories, Guelph, Ont.

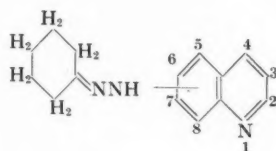
and this was identical with the cyclization product of 6-quinolyhydrazone of cyclohexanone. Dehydrogenation of 8,9,10,11-tetrahydro-IX ($R = H$) was extremely difficult. Heating it with chloroanil or with platinum catalyst at 350° did not cause any appreciable change. The pyridocarbazole (IX, $R = H$) was finally obtained by heating the tetrahydro compound with selenium at 350° . This compound (IX, $R = H$) was also synthesized from 1-phenyl-5-amino-1-benzotriazole (X) (11). In the Skraup reaction X yielded 3-phenyl-3-triazolo(*f*)-quinoline (XI) (5) which on pyrolyses at 400° gave IX ($R = H$) in 15% yield.



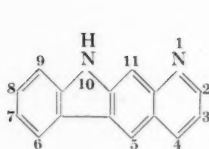
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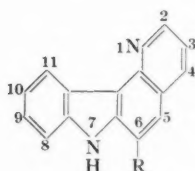
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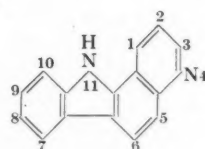
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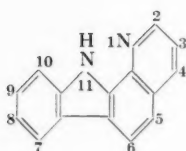
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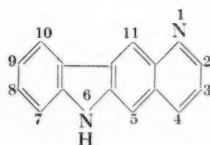
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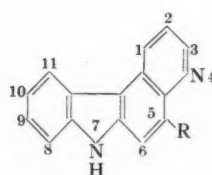
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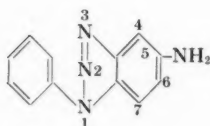
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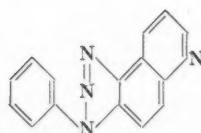
VIII



IX



X



XI

Incidentally, X can theoretically undergo the Skraup reaction in two directions to form 3-phenyl-3-triazolo(f)quinoline (XI) and (or) the linear 1-phenyl-1-triazolo(g)quinoline. That the angular compound (XI) was formed in this reaction and not the linear isomer is proved indirectly by the fact that it pyrolyzed to IX (R = H) and not to VIII.

6-Chloro-7-quinolylhydrazone of cyclohexanone underwent ring closure with difficulty and in poor yield to 6-chloro-8,9,10,11-tetrahydro-7H-pyrido(3,2-c)-carbazole (tetrahydro-V, R = Cl). The identity of the dechlorination product of 8,9,10,11-tetrahydro-V (R = Cl) and the cyclization product of 7-quinolylhydrazone of cyclohexanone showed that the 7-quinolylhydrazone undergoes ring closure to form the angular compound (tetrahydro-V, R = H). The dehydrogenation of the latter compound to V (R = H) was best accomplished by heating with chloroanil in xylene.

The linear pyridocarbazoles IV and VIII could not be synthesized from 6- and 7-quinolylhydrazones (III) because the tendency for angular compound formation could not be suppressed by blocking the reactive positions (5 and 8). However the reactivity of the 5- and 8-positions could be decreased and that of the 6- and 7-positions in III increased by hydrogenating the hetero ring. N-Benzoyl-1,2,3,4-tetrahydro-6-quinolylhydrazone of cyclohexanone was prepared and subjected to acid treatment. A mixture of 1,2,3,4,7,8,9,10-octahydro-6H-pyrido(3,2-b)- (octahydro-VIII) and 1,2,3,4,8,9,10,11-octahydro-7H-pyrido-(2,3-c)carbazole (octahydro-IX, R = H) in 30 and 18% yield respectively was obtained. These were separated by crystallization and dehydrogenated to VIII and IX (R = H) respectively by heating with platinum catalyst.

A parallel synthesis of IV from 1,2,3,4-tetrahydro-7-quinolylhydrazone of cyclohexanone (III) presented a number of obstacles. When N-*p*-toluenesulphonyl-1,2,3,4-tetrahydro-7-quinolylhydrazone of cyclohexanone was heated in aqueous acid solution or in acetic acid in the presence of sulphuric acid only black resinous material was obtained. The Fischer-indole ring closure of N-benzoyl- and N-acetyl-1,2,3,4-tetrahydro-7-quinolylhydrazones of cyclohexanone to N-benzoyl- (octahydro-N-benzoyl-IV) and N-acetyl-1,2,3,4,6,7,8,9-octahydro-10H-pyrido-(2,3-b)carbazole (octahydro-N-acetyl-IV) respectively could be accomplished by prolonged treatment with hydrochloric acid at room temperature. However attempted hydrolysis of octahydro-N-benzoyl-IV with alcoholic alkali gave only unchanged material and treatment with boiling aqueous hydrochloric acid resulted in decomposition. Octahydro-N-acetyl-IV was also sensitive to hot aqueous acids, but heating a short time with 25% hydrochloric acid resulted in deacetylation and precipitation of the hydrochloride of octahydro-IV. The dehydrogenation of octahydro-IV with platinum catalyst required initial heating at 160–170° for one or two hours before elevating the temperature to 200°. A rapid initial heating above 170° caused a minor explosion even in an atmosphere of nitrogen.

The pyridocarbazoles IV to IX inclusive, like those of the isoquinoline series (9, 10), exhibit a violet fluorescence when in dilute solution. The linear polycyclic systems are less soluble and possess higher melting points than the corresponding

angular isomers. The hydrochlorides of the pyridocarbazoles are all sparingly soluble in water.

The authors are indebted to A. E. Ledingham and R. Mills for the microanalyses.

EXPERIMENTAL

A. 11H-Pyrido(3,2-a)carbazole (VI)

To a hot solution of 7,8,9,10-tetrahydro-11H-pyrido(3,2-a)carbazole (tetrahydro-VI) (3) (0.5 gm.) in xylene (100 cc.) was added chloroanil (1.2 gm.) and the reaction mixture was heated under reflux for 24 hr. in a nitrogen atmosphere. The cooled reaction mixture was extracted with two 300 cc. portions of hot dilute hydrochloric acid (VI-HCl is not very soluble in cold water), the aqueous extract filtered, and the filtrate basified with sodium hydroxide. The white precipitate was filtered, washed, and crystallized from pyridine—yield, 0.26 gm. or 50%; m.p. 333–335°, literature (2) m.p. 335°.

B. 11H-Pyrido(2,3-a)carbazole (VII)

7,8,9,10-Tetrahydro-11H-pyrido(2,3-a)carbazole (3) (tetrahydro-VII) was dehydrogenated as in Section A—yield, 27%; m.p. 172–173°. Tetrahydro-VII could not be dehydrogenated by heating with platinum catalyst at 330° in an inert atmosphere. Clemo and Felton (2) report a melting point of 165° for VII which they obtained by dehydrogenation with palladium. Undoubtedly the low melting point is due to incomplete dehydrogenation.

C. 6-Hydrazino-5,8-dichloroquinoline

A solution of 6-amino-5,8-dichloroquinoline (7) (10 gm.) in concentrated hydrochloric acid (60 cc.) was diazotized at 0° with a solution of sodium nitrite (3.5 gm.) in water (15 cc.). The diazonium salt solution was then gradually added to a chilled solution of stannous chloride dihydrate (35 gm.) in concentrated hydrochloric acid (40 cc.), keeping the temperature below 10°. The reaction mixture was allowed to stand at approximately 7° overnight, the yellow precipitate filtered, dissolved in hot water (400 cc.), and the solution saturated with hydrogen sulphide. The tin sulphides were filtered and the filtrate basified with sodium hydroxide. The filtered precipitate was crystallized from ethanol to give light-yellow needles (6.2 gm. or 60%), m.p. 178° dec. Anal. calc. for $C_9H_7N_3Cl_2$: C, 47.37; H, 3.07; N, 18.42. Found: C, 47.78, 47.65; H, 2.84, 3.09; N, 18.20.

D. 5,8-Dichloro-6-quinolylhydrazone of Cyclohexanone

6-Hydrazino-5,8-dichloroquinoline (6.0 gm.) was suspended in ethanol (150 cc.), cyclohexanone (3.5 cc.) added, and the reaction mixture heated under reflux for one hour. The amber solution was concentrated to about 50 cc. and allowed to cool, yielding golden-yellow needles (7.5 gm. or 90%), m.p. 122–125° dec. Anal. calc. for $C_{15}H_{15}N_3Cl_2$: C, 58.43; H, 4.87; N, 13.63. Found: C, 58.37, 58.38; H, 4.78, 4.82; N, 13.61.

E. 5-Chloro-8,9,10,11-tetrahydro-7H-pyrido(2,3-c)carbazole (Tetrahydro-IX, R = Cl)

Into boiling butanol (50 cc.) dry hydrogen chloride was passed while a solution of 5,8-dichloro-6-quinolylhydrazone of cyclohexanone (4.0 gm.) in warm butanol

(200 cc.) was added through the condenser over a period of one-half hour. The resulting reaction mixture was heated under reflux for five hours while a stream of hydrogen chloride was passed in. The butanol was distilled off, the residue extracted with hot dilute hydrochloric acid, the extract filtered, and the filtrate basified. The filtered precipitate was distilled at 0.3 mm. pressure collecting the fraction boiling at 200–250° bath temperature. The distillate was crystallized from methanol and from acetone (violet fluorescence in dilute solution) to give light-yellow needles (0.65 gm. or 18%), m.p. 254–255°. Anal. calc. for $C_{15}H_{13}N_2Cl$: C, 70.18; H, 5.07; N, 10.91. Found: C, 69.76; H, 5.08; N, 10.95.

Attempts to ring close the hydrazone with aqueous acids at reflux temperatures resulted in a 1% yield of tetrahydro-IX ($R = Cl$).

F. 8,9,10,11-Tetrahydro-7H-pyrido(2,3-c)carbazole (Tetrahydro-IX, $R = H$)

(a) *From 6-quinolyldiazotone*.—6-Quinolyldiazotone of cyclohexanone was ring closed following the method of Dewar (3), (yield, 30%), m.p. 204–206°, literature, 202°.

(b) *By dechlorination of tetrahydro-IX ($R = Cl$)*.—5-Chloro-8,9,10,11-tetrahydro-7H-pyrido(2,3-c)carbazole (tetrahydro-IX, $R = Cl$) (0.12 gm.), Adams platinum catalyst (0.1 gm.), and tetralin (75 cc.) were heated under reflux for 70 hr. while the hydrogen chloride was swept out with a continuous stream of nitrogen. The tetralin was distilled off under reduced pressure and the residue distilled at 0.3 mm. pressure and 220° bath temperature. The distillate was crystallized from acetone yielding light-yellow prisms (0.05 gm. or 40%), m.p. 204–205°; mixed melting point with the product obtained in (a) gave no depression.

G. 7H-Pyrido(2,3-c)carbazole (IX, $R = H$)

(a) *From 8,9,10,11-tetrahydro-7H-pyrido(2,3-c)carbazole (tetrahydro-IX, $R = H$)*.—A mixture of tetrahydro-IX ($R = H$) (0.7 gm.) and selenium powder (3 gm.) was heated at 350–370° for two hours in an atmosphere of nitrogen and then distilled at 0.3 mm. and 250° bath temperature. The distillate on crystallization from acetone yielded light-yellow needles (0.32 gm. or 45%), m.p. 211–212°, literature (2), 211°.

(b) *From 3-phenyl-3-triazolo(f)quinoline (XI)*.—1-Phenyl-5-amino-1-benzotriazole (X) (11) was subjected to the Skraup reaction following the procedure of Fries (5). The resulting 3-phenyl-3-triazolo(f)quinoline (XI) (0.2 gm.) (m.p. 126–127°), which was obtained in 20% yield, was heated at 390–400° for 15 min. and then distilled and crystallized as in (a). The product (0.026 gm. or 15%) melted at 209–210° either alone or in admixture with that obtained in (a).

H. 7-Hydrazinoquinoline

This was prepared from 7-aminoquinoline (8) by the same method as was 6-hydrazino-5,8-dichloroquinoline (Section C). A portion of the crude hydrazine which darkened quickly on standing was crystallized from methanol to give light-yellow needles, m.p. 162–163 dec. Anal. calc. for $C_9H_9N_3$: C, 67.92; H, 5.65; N, 26.40. Found: C, 67.76, 68.12; H, 5.58, 5.51; N, 26.22, 26.67.

I. 7-Quinolyldiazotone of Cyclohexanone

Crude 7-hydrazinoquinoline (1.0 gm.) was dissolved in methanol (20 cc.),

cyclohexanone (1 gm.) was added, and the dark amber solution allowed to stand at room temperature for several hours. The precipitated orange plates (1.1 gm. or 73%) were filtered and recrystallized from methanol, m.p. 158–159°. Anal. calc. for $C_{15}H_{17}N_3$: C, 75.29; H, 7.15; N, 17.55. Found: C, 75.43, 75.37; H, 6.83, 6.97; N, 18.08, 18.12.

K. 7-Amino-6-chloroquinoline

To a solution of 7-nitro-6-chloroquinoline (4) (1.5 gm.) in concentrated hydrochloric acid (20 cc.) was added a solution of stannous chloride dihydrate (7 gm.) in concentrated hydrochloric acid (10 cc.), the reaction mixture was heated on the steam bath for one hour and cooled. The yellow precipitate was filtered, dissolved in hot water (200 cc.), and poured into excess dilute sodium hydroxide solution containing cracked ice. The amine was filtered and crystallized from benzene, yielding (0.95 gm. or 75%) white needles, m.p. 151–152°. Anal. calc. for $C_9H_7N_2Cl$: C, 60.50; H, 3.92; N, 15.69. Found: C, 60.49; H, 3.85; N, 15.41.

L. 6-Amino-5-nitroquinoline

To ethanol (100 cc.) containing ammonia (10 gm.) was added 6-chloro-5-nitroquinoline (4) (1.0 gm.) and the reaction mixture was heated in a sealed bomb at 160° for 24 hr. The reaction mixture was taken to dryness, the residue dissolved in dilute hydrochloric acid, the solution filtered, and the filtrate basified with sodium hydroxide. The precipitate was filtered, washed, dried, and crystallized from benzene or methanol to yield orange prisms (0.6 gm. or 70%), m.p. 177–178°. Anal. calc. for $C_9H_7N_3O_2$: C, 57.14; H, 3.70; N, 22.22. Found: C, 57.42, 57.52; H, 3.75, 3.42; N, 21.90.

M. 6-Chloro-8,9,10,11-tetrahydro-7H-pyrido(3,2-c)carbazole (Tetrahydro-V, R = Cl)

7-Amino-6-chloroquinoline (0.90 gm.) was converted to 7-hydrazino-6-chloroquinoline which in turn reacted with cyclohexanone by methods already described (Sections C and D). The resulting crude 6-chloro-7-quinolyldiazine of cyclohexanone was dissolved in acetic acid (15 cc.), concentrated sulphuric acid (2 cc.) was added, and the solution heated on the steam bath for one hour. The solution was poured into excess aqueous sodium hydroxide, the precipitated oily material was extracted with ether, the ether removed, and the residue distilled at 0.5 mm. pressure and 200° bath temperature. The oily distillate (0.22 gm.) which could not be induced to crystallize was dissolved in a minimum amount of hot dilute hydrochloric acid and allowed to cool. The precipitated orange needlelike crystals of the hydrochloride were filtered, dissolved in hot water, and the solution basified with sodium hydroxide. The precipitated free base was filtered and crystallized from ethyl acetate to give colorless prisms (0.10 gm. or 10%), m.p. 163–164°. Anal. calc. for $C_{15}H_{13}N_2Cl$: C, 70.18; H, 5.07; N, 10.91. Found: C, 70.48; H, 5.26; N, 11.10.

N. 8,9,10,11-Tetrahydro-7H-pyrido(3,2-c)carbazole (Tetrahydro-V, R = H)

(a) *From 7-quinolyldiazine of cyclohexanone.*—7-Quinolyldiazine of cyclohexanone (1.0 gm.) was cyclized and the resulting tetrahydro-V (R = H) purified through the hydrochloride in the same manner as described for 6-chloro-8,9,10,11-tetrahydro-7H-pyrido(3,2-c)carbazole (Section M). The product on

crystallization from dilute methanol and from dibutyl ether yielded stout brown prisms (0.55 gm. or 60%), m.p. 154–155°. Anal. calc. for $C_{15}H_{14}N_2$: C, 81.04; H, 6.34; N, 12.61. Found: C, 81.02, 81.56; H, 6.27, 6.38; N, 12.85, 12.85.

(b) *From 6-chloro-8,9,10,11-tetrahydro-7H-pyrido(3,2-c)carbazole (tetrahydro-V, R = Cl).*—Tetrahydro-V (R = Cl) (0.05 gm.) was dechlorinated catalytically by the same method as was 5-chloro-8,9,10,11-tetrahydro-7H-pyrido(2,3-c)carbazole (Section F). A few crystals were obtained which melted at 149–151° and gave no depression when mixed with the tetrahydro-V (R = H) obtained in (a).

O. 7H-Pyrido(3,2-c)carbazole (V, R = H)

8,9,10,11-Tetrahydro-7H-pyrido(3,2-c)carbazole (tetrahydro V, R = H) was dehydrogenated with chloroanil in the same manner as described in Section A. The crude product was distilled at 0.3 mm. pressure and 220° bath temperature and the distillate on crystallization from methanol and from ethyl acetate gave white prisms (yield 40%), m.p. 173–174°. Anal. calc. for $C_{15}H_{10}N_2$: C, 82.52; H, 4.62; N, 12.83. Found: C, 82.76, 81.99; H, 4.66, 4.56; N, 12.70, 12.94.

P. 6-Hydrazino-1-benzoyl-1,2,3,4-tetrahydroquinoline

A solution of 6-amino-1-benzoyl-1,2,3,4-tetrahydroquinoline (7) (11.4 gm.) in water (50 cc.) and concentrated hydrochloric acid (70 cc.) was diazotized at 0° with a solution of sodium nitrite (3.2 gm.) in water (35 cc.). The diazonium salt solution was then added portionwise to a chilled solution of stannous chloride dihydrate (40 gm.) in concentrated hydrochloric acid (60 cc.), the temperature being maintained below 5°. After it had been allowed to stand at room temperature for two hours the reaction mixture was poured into excess sodium hydroxide solution containing cracked ice. The precipitated hydrazine was filtered (or extracted with 2 liters of ether) and crystallized from benzene to give light-yellow prisms (10.0 gm. or 80%), m.p. 150–152°. Anal. calc. for $C_{16}H_{17}N_3O$: C, 71.91; H, 6.37; N, 15.72. Found: C, 72.25, 72.27; H, 6.16, 6.23; N, 15.31.

Q. 1-Benzoyl-1,2,3,4-tetrahydro-6-quinolylhydrazone of Cyclohexanone

To a solution of 6-hydrazino-1-benzoyl-1,2,3,4-tetrahydroquinoline (10.0 gm.) in methanol (100 cc.) was added cyclohexanone (10 cc.) and the solution was heated under reflux for five minutes. The precipitated almost-white prisms (11.1 gm. or 85%) were filtered from the cooled solution, washed, and dried, m.p. 200–205° dec. Anal. calc. for $C_{22}H_{25}N_3O$: C, 76.08; H, 7.20; N, 12.10. Found: C, 75.37, 75.61; H, 6.91, 6.97; N, 12.16.

R. 1,2,3,4,7,8,9,10-Octahydro-6H-pyrido(3,2-b)carbazole (Octahydro-VIII) and the Angular Isomer

1-Benzoyl-1,2,3,4-tetrahydro-6-quinolylhydrazone of cyclohexanone (10.0 gm.) was dissolved in acetic acid (100 cc.), concentrated hydrochloric acid (100 cc.) added, and the solution allowed to stand at room temperature for three days. The reaction mixture was diluted with water (50 cc.) and heated under reflux for five hours in order to hydrolyze the benzoyl group. It was then cooled and poured into excess sodium hydroxide solution containing cracked ice. The cream-colored precipitate was filtered, washed, dried, and crystallized from

benzene. The yield of the white feathery crystals (m.p. 216–217°) of octahydro-VIII was 2.0 gm. or 30%. Anal. calc. for $C_{16}H_{18}N_2$: C, 79.64; H, 7.96; N, 12.39. Found: C, 79.28, 79.82; H, 7.80, 7.85; N, 11.90.

The benzene mother liquors from the crystallization of octahydro-VIII were taken to dryness, the residual oil mixed with Adams platinum catalyst (0.1 gm.) and heated at 210–220° for five hours in an atmosphere of nitrogen. The product on distillation at 0.3 mm. pressure and 220° bath temperature and crystallization from acetone and from methanol (yield, 1.2 gm. or 18%) melted at 203–205° and gave no depression when mixed with 8,9,10,11-tetrahydro-7H-pyrido(2,3-*c*)carbazole (tetrahydro-IX, R = H) (Section F).

S. 6H-Pyrido(3,2-b)carbazole (VIII)

1,2,3,4,7,8,9,10-Octahydro-6H-pyrido(3,2-*b*)carbazole (octahydro-VIII) (0.85 gm.) was mixed with Adams platinum catalyst (0.2 gm.) and the mixture heated at 210–220° for three hours in an atmosphere of nitrogen and then at 270–280° for five hours. The product was distilled at 0.3 mm. pressure and 220° bath temperature. The distillate was mixed with fresh catalyst and re-treated as above. The final product on crystallization from acetone yielded stout yellow prisms (0.28 gm. or 30%), m.p. 282–284°. (Further heating with platinum at 300° did not change the melting point.) Anal. calc. for $C_{16}H_{18}N_2$: C, 82.51; H, 4.63; N, 12.83. Found: C, 82.37, 82.35; H, 4.75, 4.75; N, 12.69.

T. 7-Hydrazino-1-benzoyl-1,2,3,4-tetrahydroquinoline

This was prepared from 7-amino-1-benzoyl-1,2,3,4-tetrahydroquinoline (7) by the same method as was the 6-isomer (Section P). Crystallization from methanol yielded (85%) small white needles, m.p. 115–120°, which decomposed rapidly at room temperature. Anal. calc. for $C_{16}H_{17}N_3O$: C, 71.91; H, 6.37; N, 15.72. Found: C, 72.46, 72.64; H, 6.34, 6.20; N, 14.51.

U. 1-Benzoyl-1,2,3,4-tetrahydro-7-quinolylhydrazone of Cyclohexanone

This was prepared from 7-hydrazino-1-benzoyl-1,2,3,4-tetrahydroquinoline by the same method as was the 6-isomer (Section Q). It crystallized from methanol as light-yellow prisms (yield, 80%), m.p. 205–207°. Anal. calc. for $C_{22}H_{26}N_2O$: C, 76.08; H, 7.20; N, 12.10. Found: C, 75.98, 76.01; H, 7.08, 7.54; N, 11.70.

V. 1,2,3,4,6,7,8,9-Octahydro-1-benzoyl-10H-pyrido(2,3-b)carbazole (Octahydro-1-benzoyl-IV)

To a solution of 1-benzoyl-1,2,3,4-tetrahydro-7-quinolylhydrazone of cyclohexanone (8.0 gm.) in acetic acid (50 cc.) was added concentrated hydrochloric acid (50 cc.) and the solution was allowed to stand at room temperature for five days. The resulting dark solution was diluted with water, the precipitate filtered, washed, dried, and crystallized from benzene and from ethanol to yield white needlelike crystals (5.5 gm. or 70%), m.p. 242–243°. Anal. calc. for $C_{22}H_{22}N_2O$: C, 80.00; H, 6.67; N, 8.48. Found: C, 80.06, 80.07; H, 6.60, 6.57; N, 8.86.

Attempted hydrolysis of this compound with aqueous acids resulted in decomposition; it was recovered unchanged from boiling alcoholic alkali.

W. 1-Acetyl-1,2,3,4-tetrahydro-7-quinolylhydrazone of Cyclohexanone

7-Hydrazino-1-acetyl-1,2,3,4-tetrahydroquinoline was prepared in 60% yield

from 7-amino-1-acetyl-1,2,3,4-tetrahydroquinoline (7) by the same method as was 6-hydrazino-1-benzoyl-1,2,3,4-tetrahydroquinoline (Section P). The resulting oily 7-hydrazino-1-acetyl-1,2,3,4-tetrahydroquinoline was converted to the hydrazone with cyclohexanone (Section Q). The almost-white prisms (90% yield) melted at 153–157°. Anal. calc. for $C_{17}H_{23}N_3O$: C, 71.58; H, 8.07; N, 14.74. Found: C, 71.53, 71.26; H, 7.98, 8.04; N, 14.69.

X. 1,2,3,4,6,7,8,9-Octahydro-10H-pyrido(2,3-b)carbazole (Octahydro-IV)

1-Acetyl-1,2,3,4-tetrahydro-7-quinolyldiazone of cyclohexanone (4.5 gm.) was dissolved in concentrated hydrochloric acid (25 cc.), water (25 cc.) was added, and the resulting reddish solution allowed to stand for three days at room temperature. To the reaction mixture containing precipitated semisolid was added concentrated hydrochloric acid (30 cc.) and the solution was heated under reflux for 15 min. and on the steam bath for 45 min. (The use of more dilute acid or prolonged heating causes excessive decomposition.) The precipitated octahydro-IV-hydrochloride was filtered from the cooled reaction mixture, dissolved in hot water (1 liter), and the solution basified with sodium hydroxide. The crude octahydro-IV (2.5 gm. or 70%) was filtered, washed, dried, and quickly crystallized from benzene. This yielded white needles which darkened on standing, m.p. 175–176°. Anal. calc. for $C_{16}H_{18}N_2$: C, 79.64; H, 7.96; N, 12.39. Found: C, 79.09, 78.98; H, 7.54, 7.55; N, 11.83.

No octahydro-V ($R = H$) could be found in the mother liquors from octahydro-IV and in those from its hydrochloride.

Y. 6,7,8,9-Tetrahydro-10H-pyrido(2,3-b)carbazole (Tetrahydro-IV)

1,2,3,4,6,7,8,9-Octahydro-10H-pyrido(2,3-b)carbazole (octahydro-IV) (2.5 gm.) was mixed with Adams platinum catalyst (0.5 gm.) and heated in an atmosphere of nitrogen at 160–170° for two hours and at 200° for two hours. The product was distilled at 0.3 mm. pressure and 210° bath temperature and the distillate crystallized from ethyl acetate or methanol to yield yellow prisms (1.7 gm. or 68%), m.p. 232–233°. Anal. calc. for $C_{15}H_{14}N_2$: C, 81.04; H, 6.34; N, 12.61. Found: C, 81.71, 81.76; H, 6.19, 6.31; N, 12.34.

Z. 10H-Pyrido(2,3-b)carbazole (IV)

6,7,8,9-Tetrahydro-10H-pyrido(2,3-b)carbazole was dehydrogenated with chloroanil as described in Section A. The crude product (yield, 10%) on crystallization from methanol yielded yellow needles, m.p. 245–246°. Anal. calc. for $C_{15}H_{10}N_2$: C, 82.51; H, 4.63. Found: C, 82.29; H, 4.77.

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THE NITRATION OF SOME QUINOLINE DERIVATIVES¹

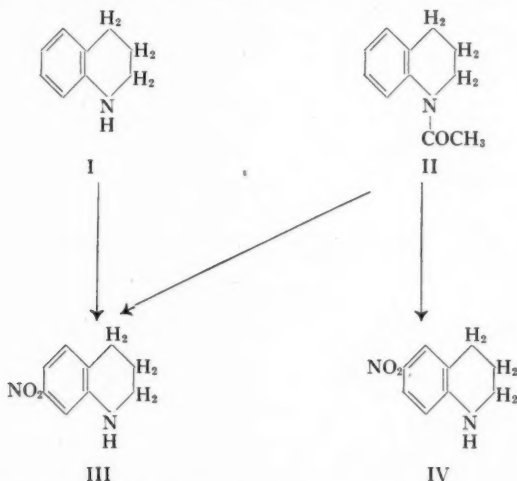
BY MARSHALL KULKA AND R. H. F. MANSKE

ABSTRACT

Three compounds, namely 1,2,3,4-tetrahydroquinoline, N-acetyl-1,2,3,4-tetrahydroquinoline, and 5,8-dichloroquinoline, have been nitrated and the nitration products orientated through reduction studies.

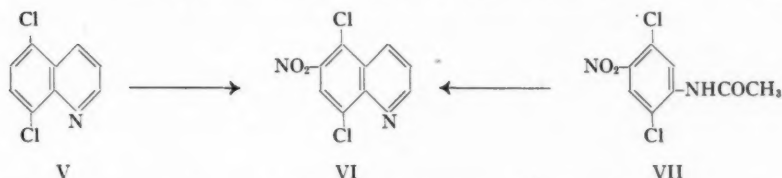
A number of nitro- and aminoquinoline derivatives were required as starting materials in the synthesis of polycyclic systems containing the quinoline ring (7). Although many nitroquinolines have been prepared by direct nitration or by utilizing the Skraup reaction, a few of the required ones were heretofore unknown and others lacked preparative methods. This paper describes the nitration of 1,2,3,4-tetrahydroquinoline (I), N-acetyl-1,2,3,4-tetrahydroquinoline (II), and 5,8-dichloroquinoline (V) and the orientation of the nitration products.

A comparison of the properties of quinoline and 1,2,3,4-tetrahydroquinoline (I) shows that the two behave differently in nitration reactions. While the former is attacked mainly at the 5- and 8-positions (3) the latter yields 7-nitro-1,2,3,4-tetrahydroquinoline (III) exclusively in the presence of nitric acid. v. Braun, Grabowski, and Rawicz (2) first nitrated I and obtained a single nitro compound melting at 90° (N-benzoyl derivative, m.p. 141°). In our hands this nitration yielded a pure compound melting at 63° (N-benzoyl derivative, m.p. 154°). That this was 7-nitro-1,2,3,4-tetrahydroquinoline (III) was shown by catalytic reduction to 7-amino-1,2,3,4-tetrahydroquinoline and comparison of the latter with an authentic sample prepared from 7-nitroquinoline (1) by reduction.



¹ Manuscript received May 8, 1952.

Contribution from the Dominion Rubber Company Limited Research Laboratories, Guelph, Ont.



The nitration of 1-acetyl-1,2,3,4-tetrahydroquinoline (II), followed by acid hydrolysis, yielded a mixture of 6- (IV) and 7-nitro-1,2,3,4-tetrahydroquinoline (III) in the ratio of approximately two to one. The identity of the 6-nitro derivative (IV) was established by catalytic reduction to 6-amino-1,2,3,4-tetrahydroquinoline and comparison of this with an authentic sample prepared from 6-nitroquinoline (6) by reduction. That 7-nitro-1,2,3,4-tetrahydroquinoline (III) is one of the nitration products of II is surprising in view of the fact that the treatment of 1,2,3,4-tetrahydroquinoline with excess nitrous acid followed by hydrolysis yields a mixture of 6- (IV) and 8-nitro-1,2,3,4-tetrahydroquinoline (9).

5,8-Dichloroquinoline (V), which was required for nitration studies, was prepared from 2,5-dichloroaniline in high yield by the Skraup reaction (4). It (V) was found extremely resistant to nitration. A solution of nitric acid and concentrated sulphuric acid or oleum did not attack it at room temperature. It was finally nitrated in oleum at 60° for 45 hr. to yield 6-nitro-5,8-dichloroquinoline (VI). That the nitration product was 6-nitro- (VI) and not 7-nitro-5,8-dichloroquinoline was shown by comparison with VI synthesized unambiguously from 2,5-dichloro-4-nitroacetanilide (VII) (8) by the Skraup reaction.

Attempts to synthesize the isomeric 7-nitro-5,8-dichloroquinoline were not successful. 2,5-Dichloro-3-nitroaniline required for this purpose could not be prepared by direct nitration of 2,5-dichloroaniline in excess sulphuric acid. 6-Amino-7-nitro-5,8-dichloroquinoline, which should yield the desired compound on deamination, was not obtained when 6-acetylamino-5,8-dichloroquinoline was subjected to various nitration experiments.

The authors are indebted to A. E. Ledingham and R. Mills for the microanalyses.

EXPERIMENTAL

7-Nitro-1,2,3,4-tetrahydroquinoline (III)

This was prepared by the nitration of tetrahydroquinoline following the procedure of v. Braun, Grabowski, and Rawicz (2) who report a melting point of 90°. The product after crystallization from methanol melted at 60–61° (yield 65%) and repeated recrystallizations from petroleum ether, dibutyl ether, and methanol did not raise the melting point above 62–63°. Anal. calc. for C₉H₁₀N₂O₂: C, 60.67; H, 5.62; N, 15.72. Found: C, 60.90, 60.57; H, 5.32, 5.42; N, 16.13.

N-Acyl Derivatives of 7-Nitro-1,2,3,4-tetrahydroquinoline

These were prepared by adding excess acyl halide or anhydride to a solution of 7-nitro-1,2,3,4-tetrahydroquinoline (III) (10 gm.) in pyridine (10 cc.), heating

* All melting points are corrected.

on the steam bath for 15 min., pouring into cold water, filtering, and crystallizing from ethanol.

(a) *N-Benzoyl-7-nitro-1,2,3,4-tetrahydroquinoline*, yield, 14.0 gm. or 90%, m.p. 153–154°, literature (2) m.p. 141°. Anal. calc. for $C_{16}H_{14}N_2O_3$: C, 68.10; H, 4.96; N, 9.93. Found: C, 68.18, 67.84; H, 5.14, 4.90; N, 10.15.

(b) *N-Acetyl-7-nitro-1,2,3,4-tetrahydroquinoline*, white needlelike prisms, m.p. 102–103°, yield, 92%. Anal. calc. for $C_{11}H_{12}N_2O_3$: C, 60.00; H, 5.45; N, 12.73. Found: C, 59.76, 59.62; H, 5.23, 5.10; N, 12.78.

(c) *N-p-Toluenesulphonyl-7-nitro-1,2,3,4-tetrahydroquinoline*, colorless prisms, m.p. 164–165°, yield, 80%. Anal. calc. for $C_{16}H_{16}N_2O_4S$: N, 8.43. Found: N, 8.59, 8.41.

7-Amino-N-benzoyl-1,2,3,4-tetrahydroquinoline

7-Nitro-N-benzoyl-1,2,3,4-tetrahydroquinoline (8.0 gm.) was suspended in a warm solution of ethyl acetate (150 cc.) and methanol (50 cc.), Adams platinum catalyst (0.05 gm.) was added, and the reaction mixture shaken under 50 lb. of hydrogen until hydrogenation was complete. The catalyst and the solvent were removed and the residue crystallized from methanol—yield, 6.3 gm. or 89%, light-yellow prisms, m.p. 140–141°. Anal. calc. for $C_{16}H_{16}N_2O$: C, 76.20; H, 6.35; N, 11.11. Found: C, 76.55, 76.42; H, 6.35, 6.25; N, 11.40.

7-Amino-N-acetyl-1,2,3,4-tetrahydroquinoline

7-Nitro-N-acetyl-1,2,3,4-tetrahydroquinoline was catalytically reduced as above, colorless prisms from benzene, m.p. 77–78°, yield, 94%. Anal. calc. for $C_{11}H_{14}N_2O$: C, 69.41; H, 7.37; N, 14.73. Found: C, 69.95, 69.55; H, 7.52, 7.37; N, 14.57.

7-Amino-N-p-toluenesulphonyl-1,2,3,4-tetrahydroquinoline

7-Nitro-N-p-toluenesulphonyl-1,2,3,4-tetrahydroquinoline was catalytically reduced as above, colorless prisms from methanol, m.p. 109–110°, yield, 90%. Anal. calc. for $C_{16}H_{18}N_2O_2S$: N, 9.27. Found: N, 9.37, 9.33.

7-Amino-1,2,3,4-tetrahydroquinoline

(a) *From 7-nitroquinoline*.—To a solution of 7-nitroquinoline (1.0 gm.) in acetic acid (25 cc.) was added Raney nickel catalyst (about 1 gm.) and the reaction mixture was heated at 60–70° for four hours under 1200 lb. hydrogen pressure. The solvent and catalyst were removed and the residue distilled at 0.5 mm. pressure and 150° bath temperature. The colorless distillate on crystallization from benzene–petroleum ether yielded (0.17 gm. or 20%) colorless prisms, m.p. 60–61°. Anal. calc. for $C_9H_{12}N_2$: C, 72.97; H, 8.10; N, 18.90. Found: C, 73.18, 72.79; H, 7.71, 7.98; N, 18.66.

(b) *From 7-nitro-1,2,3,4-tetrahydroquinoline*.—To a solution of 7-nitro-1,2,3,4-tetrahydroquinoline (1.0 gm.) and ethanol (75 cc.) was added Adams platinum catalyst (0.05 gm.) and the reaction mixture was shaken under 40 lb. of hydrogen pressure for one hour. The product was worked up as in (a), m.p. 60–61°; literature (2) 60°, yield quantitative, mixed with the product obtained in (a) gave no depression in melting point.

Nitration of N-Acetyl-1,2,3,4-tetrahydroquinoline

N-Acetyl-1,2,3,4-tetrahydroquinoline (II) (50 gm.) was dissolved with stirring

in concentrated sulphuric acid (250 cc.) at 0°. To this was added portionwise with stirring powdered potassium nitrate (29 gm.) over a period of one-half hour the temperature being maintained at 0°. After it had been stirred at 0° for an additional hour the solution was added to cracked ice (1.5 kgm.). The oily precipitate was extracted with benzene, washed with water, and the solvent removed from the extract. The residual oil was dissolved in concentrated hydrochloric acid (75 cc.), water (75 cc.) added, and the reaction mixture heated under reflux for one hour. On cooling an orange hydrochloride precipitated; this was filtered, dissolved in boiling water, and the solution was basified with sodium hydroxide. An orange precipitate was formed which when crystallized from methanol melted at 60–61°, yield 10 gm. or 20%; mixed melting point with 7-nitro-1,2,3,4-tetrahydroquinoline (III) gave no depression.

The acid filtrate from the hydrochloride of III was basified with sodium hydroxide, the orange precipitate was filtered, washed, and crystallized from methanol. The yield of the dark orange prisms with a metallic luster of 6-nitro-1,2,3,4-tetrahydroquinoline (IV) from three successive crops was 19 gm. or 37%, m.p. 161–162°, literature (9) m.p. 163–164°.

N-Benzoyl-6-nitro-1,2,3,4-tetrahydroquinoline

This was prepared from 6-nitro-1,2,3,4-tetrahydroquinoline (IV) and benzoyl chloride by the same method as the 7-isomer (see above); crystallized from ethanol, white needles, m.p. 134–135°, yield, 88%. Anal. calc. for $C_{16}H_{14}N_2O_3$: C, 68.10; H, 4.96; N, 9.93. Found: C, 68.31, 68.51; H, 5.23, 4.81; N, 10.16.

N-Benzoyl-6-amino-1,2,3,4-tetrahydroquinoline

6-Nitro-N-benzoyl-1,2,3,4-tetrahydroquinoline was hydrogenated in the presence of platinum catalyst by the same method as the 7-isomer (see above); light-orange needles from methanol, m.p. 174–175°, yield, 90%. Anal. calc. for $C_{16}H_{16}N_2O$: C, 76.20; H, 6.35; N, 11.11. Found: C, 76.22, 76.22; H, 6.59, 6.31; N, 11.02.

6-Amino-1,2,3,4-tetrahydroquinoline

(a) *From 6-nitroquinoline.*—6-Nitroquinoline was hydrogenated in the same way as was 7-nitroquinoline (see above), yield, 35%, white plates from benzene which darkened on exposure to air, m.p. 93–94°, literature (5) m.p. 97°.

(b) *From 6-nitro-1,2,3,4-tetrahydroquinoline (IV).*—IV was hydrogenated in the same way as was 7-nitro-1,2,3,4-tetrahydroquinoline (III) (see above); yield, 80%, m.p. 93–94°, mixed melting point with that obtained in (a) gave no depression.

5,8-Dichloroquinoline (V)

To a solution of 60% oleum (240 gm.) and concentrated sulphuric acid (240 gm.) was added nitrobenzene (160 gm.) and the reaction mixture was heated on the steam bath for five hours. The cooled solution was then added to water (160 cc.). To the resulting solution was added 2,5-dichloroaniline (162 gm.) and glycerol (240 cc.), the reaction mixture was heated under reflux for five hours, cooled, and poured into 4 liters of cold water. The tarry material was filtered off, the filtrate basified with sodium hydroxide, the precipitate filtered, washed, and crystallized from methanol (charcoal). The yield of almost-white crystals from

two successive crops was 144 gm. or 73%, m.p. 97–98°, literature (4) m.p. 98°.

6-Nitro-5,8-dichloroquinoline (VI)

(a) *By nitration.*—To a solution of 60% oleum (200 cc.), concentrated sulphuric acid (150 cc.), and 5,8-dichloroquinoline (V) (75 gm.) was added powdered potassium nitrate (50 gm.) portionwise with stirring over a period of four hours, the temperature being maintained at 55–60°. Then the reaction mixture was heated at 55–60° for 45 hr., cooled, and poured cautiously into cracked ice (2 kgm.) and water (3 liters). The acid solution was filtered (6-nitro-5,8-dichloroquinoline is insoluble in cold dilute acid), the precipitate washed, dried, and crystallized from methanol, yield, 51 gm. or 55%, light-yellow needles, m.p. 135–136°. There was no depression in melting point when this was mixed with the product obtained in (b). Anal. calc. for $C_9H_4Cl_2N_2O_2$: C, 44.44; H, 1.64; N, 11.51. Found: C, 44.89, 44.64; H, 1.82, 1.83; N, 10.91, 10.71.

(b) *By the Skraup reaction.*—2,5-Dichloro-4-nitroacetanilide (VII) (3.0 gm.), arsenic acid (3.0 gm.), glycerol (5 cc.), and concentrated sulphuric acid (4 cc.) were heated under reflux for three hours. The cooled reaction mixture was poured into water, the washed and dried black precipitate was distilled at 0.3 mm. pressure and 200° bath temperature, and the distillate crystallized from methanol. The yield of 6-nitro-5,8-dichloroquinoline was 0.65 gm. or 20%, m.p. 135–136°.

6-Amino-5,8-dichloroquinoline

To a solution of stannous chloride dihydrate (85 gm.) in concentrated hydrochloric acid (100 cc.) was added with stirring 6-nitro-5,8-dichloroquinoline (30 gm.) in five portions with cooling, the temperature being kept below 70°. Then the reaction mixture was heated on the steam bath for one hour, cooled, and filtered. The precipitate was suspended in hot water (1.5 liters), saturated with hydrogen sulphide, the tin sulphides were filtered off, and the filtrate basified with sodium hydroxide. The cream-colored precipitate was filtered, washed, dried, and crystallized from benzene, m.p. 136–137°, yield 18.0 gm. or 70%. Anal. calc. for $C_9H_6N_2Cl_2$: C, 50.70; H, 2.81; N, 13.14. Found: C, 50.66, 50.80; H, 2.67, 2.75; N, 12.96. Acetylation with acetic anhydride in benzene yielded *6-acetylamino-5,8-dichloroquinoline*, white needles, m.p. 211–212°. Anal. calc. for $C_{11}H_8Cl_2N_2O$: C, 51.76; H, 3.14; N, 10.99. Found: C, 51.76, 51.77; H, 3.34, 3.08; N, 10.50.

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NOTE

Optical Stability in Bipyridyl Compounds

In a recent paper (1) the resistance to racemization of a variety of compounds of the substituted diphenyl type was reported, one of the compounds investigated being 1-methyl-3,3'-dicarbomethoxy-2,2'-bipyridinium iodide (I). Since only a small amount of (I) was available at that time, it was stated only that the compound racemized very readily compared to the others investigated, and no quantitative data were reported. We have reinvestigated this substance, determined its half life at two different temperatures, and calculated the activation energy for racemization.

Racemization experiments were carried out in methyl cellosolve at 50°C. and 65°C. The half lives at these temperatures were 19.3 hr. and 3.3 hr., from which the energy of activation was calculated to be about 25,000 cal. This ready racemization supports the view that a nitrogen atom at the ortho position should have less blocking effect than a carbon atom with an attached hydrogen, and the value for the energy of activation is of the correct order of magnitude for easily-racemizable compounds of the diphenyl type (2).

EXPERIMENTAL

The compound (I) was prepared as described before, and was isolated as light yellow plates, m.p. (corr.) 136–137°C. The racemization experiments were carried out in 2 dm. jacketed polarimeter tubes using the sodium *D* line as the light source, and the concentration used was 0.539 gm. per 100 cc. solvent. Readings were taken at 50°C. and 65°C. until slight decomposition had deepened the original yellow color of the solutions to a point where accurate readings were no longer possible. A second series of readings on fresh samples agreed with the first within the limit of experimental error.

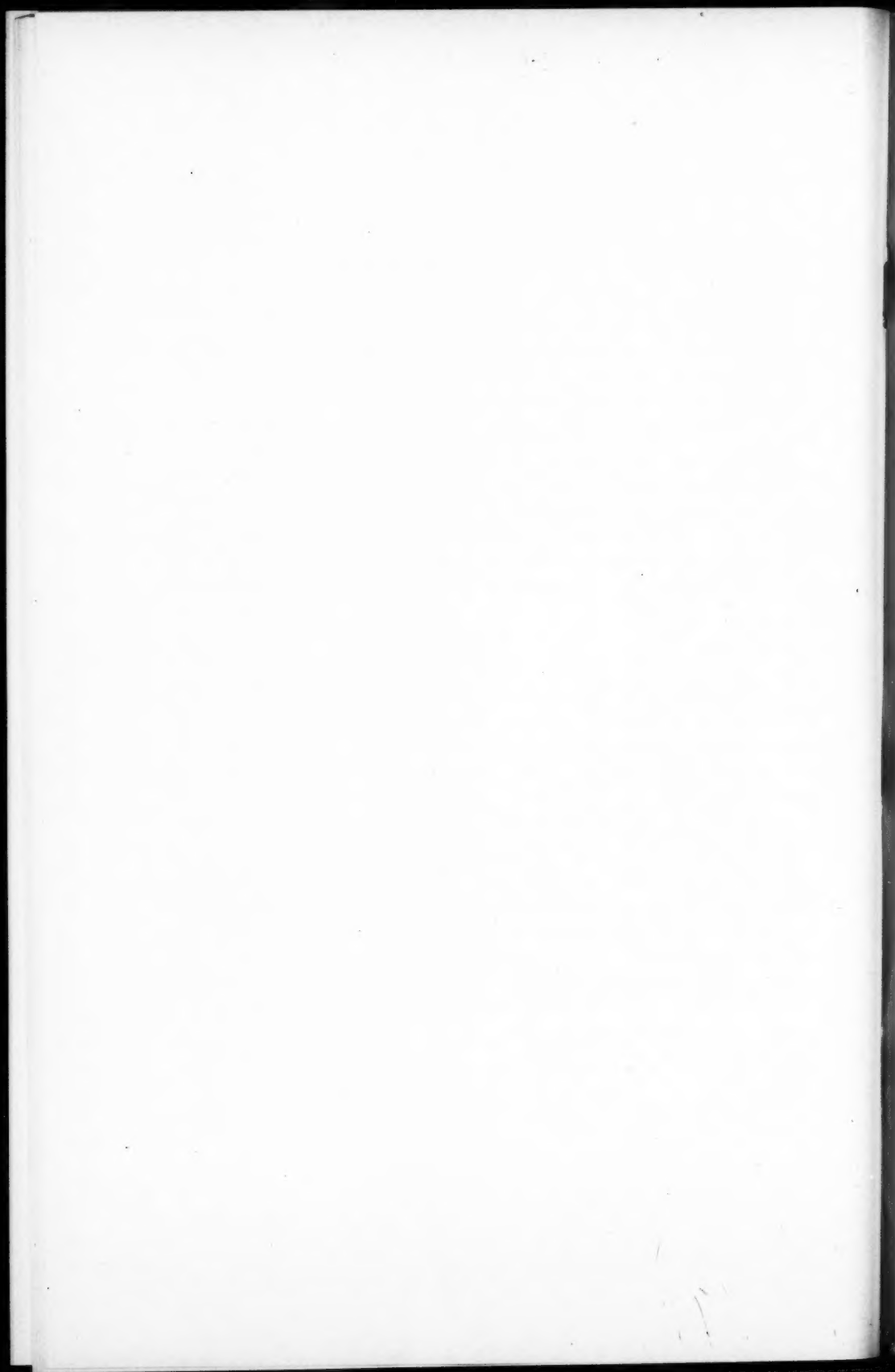
We wish to correct an error in the previous paper (1, p. 31) in which the specific rotation of the bromocamphor sulphonate salt of (I) was reported as having a high negative value. We suspected this to be incorrect, and a redetermination of the specific rotation gave values of $[\alpha]_{5461}^{25^\circ} = +45.7^\circ$, $[\alpha]_{5780}^{25^\circ} = +37.6^\circ$, for $c = 0.967$ in methanol.

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RECEIVED FEBRUARY 21, 1952.
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